STUDIES ON THE RELATIONSHIP BETWEEN VITAMIN B12 and CYANIDE IN THE AETIOLOGY OF TOBACCO AMBLYOPIA AND RELATED CONDITIONS.

by

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Being a Thesis Submitted for The Degree of M.D. of Glasgow University.

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The association between tobacco smoking and a form of amblyopia has been recognised for over one hundred years. The disease characteristically affects elderly men affects both eyes unequally and does not progress to complete blindness. The patient typicall has poor vision both for near and distance, centro-caecal scotomas larger for colour than white targets in the field of vision and an acquired colour blindness of each eye. Recent additions to the world literature on the subject have postulated an abnormality of the vitamin B12 cyanide relationship as being the underlying aetiological feature and that tobacco amblyopia is a member of a group of diseases which includes Leber's hereditary optic atrophy, the optic neuropathy of pernicious anaemia, the optic neuropathy of diabetes and some forms of tropical nutritional amblyopia.

This study reports the findings in 65 patients diagnosed as suffering from tobacco amblyopia which have been collected over a period of three years in the Western Regional Hospital Board area. The patients showed a marked reduction in visual acuity by the time they have reported for treatment.

It was found that the duration of symptoms did not influence the initial visual acuity but those patients who came under treatment early had the greatest improvement in visual acuity The Farnsworth Munsell Hundred Hue test was chosen as one of the major parameters evaluating new patients prior to and whilst on therapy. As the Hundred Hue test error scores obtained in the untreated tobacco amblyopes were higher than previously recorded analyses, an investigation to establish the validity of such error scores was carried out. It was found that with error scores above 600 it was better to take the average of several readings rather than the result of one test. In untreated tobacco amblyopia the Farnsworth Munsell Hundred Hue test result correlated well with visual acuity, and tended to do so with patient age. There was no significant correlation between the Farnsworth Munsell Hundred Hue test result and duration of symptoms, serum vitamin B12 concentration, or serum folate concentration. The incidence of the disease increases in a positive manner with age, reaching a peak in the 70-80 years age group. The mean duration of symptoms prior to seeking advice was 6 months and it was found that age played no part in determining when a patient presented for treatment.

Patients were examined for evidence of avitaminosis B12 by having serological estimations of vitamin B12 and estimations of vitamin B12 absorption carried out. Serum vitamin B12 concentrations were found to be lower in tobacco amblyopia than in the general smoking and non-smoking populations. The serum vitamin B12 concentration correlated well with tobacco consumption, the Schilling test less well so, and the Xylose absorption test poorly so. In the untreated tobacco amblyope the serum folate concentration tended to correlate with tobacco consumption. Patients with tobacco amblyopia in the presence of frank Addisonian Pernicious Anaemia exhibited higher serum folate concentrations than those patients without pernicious anaemia.

As cyanide is volatile investigations into its metabolism is to be directed to its detoxication products namely thiocyanate. Although tobacco amblyopes smoke more tobacco than non-amblyopic subjects, their serum thiocyanate concentrations are lower than those of non-amblyopic smokers and tend to resemble the concentrations found in non-smokers. These reduced concentrations, on treatment with hydroxocobalamin, tend to revert towards the higher concentrations found in the

non-amblyopic smokers. Associated with this rise in the blood there is an increased excretion of thiocyanate in the urine. A significant negative correlation was found to esixt between the plasma cyanide and the serum vitamin B12 concentrations and between the plasma thiocyanate and the renal clearance of thiocyanate in untreated tobacco amblyopia.

The keystone in therapy up till 10 years ago had been abstinence from the tobacco habit. With the growing awareness of the part played by malnutrition in this disease, preparations of vitamin B12 were used in this analysis. Of the two preparations used, hydroxocobalamin was quickly found to be superior to cyanocobalamin. The mean period that patients remained under observation was nineteen months. The majority (40 in number) had a visual acuity of 6/12 (Snellen) or better. Of the fifteen patients who had a poor restoration of vision, four were undergoing their second attack of the disease. The progress of the disease whilst on therapy was also observed by following the alteration in colour discrimination. It was found that the Farnsworth Munsell Hundred Hue test results fitted an exponential curve equation.

The rate of improvement in uncomplicated tobacco amblyopia treated with hydroxocobalamin was as good as that treated by abstinence from tobacco.

Leber's Hereditary Optic Atrophy is an inherited disease which primarily attacks the young adult. Abnormalities in cyanide detoxication products, similar to those found in tobacco amblyopia, were found in such patients. These changes underwent similar alteration on treatment with hydroxocobalamin as had been demonstrated in tobacco amblyopia and were associated with improvement in visual acuity and visual field.

The ocular features of patients suffering from the optic neuropathy of pernicious anaemia who smoked, were identical to those found in patients suffering from tobacco amblyopia. Accordingly, it is felt that the diagnosis of optic neuropathy of pernicious anaemia be reserved for those non-smoking pernicious anaemia patients. Three case histories are examined which reveal the superiority of hydroxocobalamin therapy over cyanocobalamin in this condition. In one patient the visual defect commenced after treatment with

cyanocobalamin for the haematological disorder had been continued for some time.

It is difficult in the light of present knowledge to explain the rise in thiocyanate concentrations in body fluids following a hydroxocobalamin therapy, unless a hitherto unknown mechanism sited in the kidney is postulated. It would seem that the principle biochemical defect in tobacco amblyopia would lie in the preparation of a suitable sulphur donor. In the preparation of such a donor coenzyme vitamin B12 is essential.

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....."I WILL SUMMARILY REMARKE THE HURTS THAT TOBACCO INFERRETH, IF IT BE USED CONTRARY TO THE ORDER AND WAY I HAVE SET DOWNE. IT DRIETH THE BRAINE, DIMMETH THE SIGHT, VITIATETH THE SMELL, DULLETH AND DEJECTETH BOTH THE APPETITE AND STOMACK, DESTROYETH THE CONCOCTION, DEPRAVETH THE HUMOURS AND SPIRITS, CORRUPTETH THE BREATH, INDUCETH A TREMBLING OF THE LIMBS, EXTICATETH THE WIND PIPE, LUNGS AND LIVER, ANNOYETH THE MILS, SCORCHETH THE HEART".....

THOMAS VENNER

Doctor of Physick

1650

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INTRODUCTION

It is universally recognised that tobacco amblyopia is a disease, almost exclusively, of the elderly pipe smoking male. It is the impression that, although the tobacco consumption has steadily risen since the turn of the century, the reported number of cases of tobacco amblyopia has fallen. This is in contrast to the parallelism accorded to the alleged cigarette smoking - lung cancer relationship. Assuming that the incidence of tobacco amblyopia has apparently gone down while exposure to its presumed aetiologic agent has risen, other contributory explanations must be sought.

Why does only a small proportion of pipe smokers develop the disease? What factors cause the disease and how do they alter with treatment? An attempt to answer these questions, using the in-patient and out-patient data of a study into the clinical features of the disease, is contained in this thesis. The data has been arranged under the following headings:-

1. A historical background.
2. An analysis of the means to determine the presence and severity of the disease. (Ophthalmological investigation).

3. Nutritional factors.

4. The toxic factor in tobacco.

5. The response to therapy.

6. Leber's Hereditary Optic Atrophy. Optic Neuropathy of Pernicious Anaemia.

7. Conclusions.

SUMMARY

Chapter I. Historical Background

Tobacco amblyopia is a disease of the elderly pipe-smoking male. Females may also contract the disease if sufficiently exposed to the toxic agent. Characteristi: :cally the disease is bilateral, but unequally distributed between the two eyes. The visual acuity is depressed and a glittering mist obscures objects except when viewed in twilight. A centro-caecal scotoma, larger for colours than white, is present in the field of vision, and is accompanied by an acquired colour blindness.

Tobacco amblyopia may be associated with a variety of systemic diseases, in particular diabetes, pernicious anaemia and digestive disorders. This has led to the belief that such patients are more liable to tobacco amblyopia because of a deficiency in an essential substance.

The keystone in treatment of tobacco amblyopia has been abstinence from the tobacco habit and has been in vogue from the last century. Over the past thirty years

however, the importance of malnutrition has been stressed by various authors, in particular of deficiency in vitamin B12.

Within the past eight years much new work has postulated the probable aetiology as being a disturbance in the cyanide - vitamin B12 relationship. This has led to the proposal that tobacco amblyopia is a member of a group of diseases which includes Leber's Hereditary Optic Atrophy, the Optic Neuropathy of Pernicious Anaemia, the Optic Neuropathy of Diabetes, and some forms of Tropical Nutritional Amblyopia, and in each of which a disturbance of the above relationship is present.

Chapter II. Ophthalmological Investigation

In this chapter the ophthalmic methods of investigating the visual loss found to accompany tobacco amblyopia are dealt with.

By the time they report for treatment the patients have a marked reduction in visual acuity, centro-caecal scotomas in the field of vision for each eye, and a gross

upset in colour discrimination being present. The duration of symptoms did not influence the initial visual acuity, but those patients who came under treatment early had the greatest improvement in visual acuity.

The Farnsworth Munsell 100 Hue test was chosen as one of the major parameters in evaluating individual patients prior to, and whilst on therapy. As the 100 Hue test error scores obtained in the untreated tobacco amblyopes were higher than previously recorded analyses, an investigation to establish the validity of such error scores was carried out.

In untreated tobacco amblyopia the Farnsworth Munsell 100 Hue test result correlated well with visual acuity and tended to do so with patient age. There was no significant correlation between the Farnsworth Munsell Hundred Hue test result and duration of symptoms, serum vitamin B12 concentration or serum folate concentration.

The incidence of the disease increases in a positive manner with age, reaching a peak in the 70-80 years age group, thereafter declining in frequency. The

mean duration of symptoms prior to seeking advice was 6 months and it was found that age played no part in determining when a patient presented for treatment.

Chapter III. Nutritional Factors.

In this chapter attention has been concentrated on the detection of avitaminosis B12 and the relationship of the findings to tobacco consumption. The patients were examined for coincident disease, serological examinations were carried out for total vitamin B12 and folate concentrations of the blood. Examination into vitamin B12 absorption were carried out on the patients on an in-patient basis. Liver function tests were performed to screen the patients for defective hepatic storage of vitamin B12.

Serum vitamin B12 concentrations were found to be lower in tobacco amblyopia than in the general smoking and non-smoking populations. The serum vitamin B12 concentration correlated well with tobacco consumption, the Schilling test less well so, and the Xylose absorption test poorly so.

The serum folate concentration was also investigated

and a significant proportion of the patients exhibited low concentrations of folate. In the untreated tobacco amblyope the serum folate concentration tended to correlate with tobacco consumption. Patients with tobacco amblyopia in the presence of frank Addisonian Pernicious Anaemia exhibited higher serum folate concentrations than those patients without pernicious anaemia. Age played no part in determining the serum folate concentration.

Chapter IV. The Toxic Factor in Tobacco.

In this chapter the presence of cyanide in tobacco smoke and its metabolic effects are reviewed. As cyanide is volatile investigation of its metabolism has to be directed to its detoxication products. Attention has been concentrated on thiocyanate levels in the blood and urine.

Although tobacco amblyopes smoke more tobacco than non-amblyopic subjects, their serum thiocyanate concentrations are lower than those of non-amblyopic smokers and tend to resemble the concentrations found in non-smokers. These reduced concentrations, on treatment with hydroxocobalamin, tend to revert towards the higher concentrations found in the

non-amblyopic smokers. Associated with this rise in the blood there is an increased excretion of thiocyanate in the urine and in some patients a diuresis occurs. The negative relationship between serum vitamin B12 concentration and urinary thiocyanate excretion, and the positive relationship between plasma cyanide and plasma thiocyanate found by earlier workers in healthy smokers were not confirmed in patients suffering from tobacco amblyopia. Only a tendency towards such relationships was observed. A significant negative correlation was found to exist between the plasma cyanide and the serum vitamin B12 concentrations, and between the plasma thiocyanate and the renal clearance of thiocyanate in untreated tobacco amblyopia.

Chapter V. The Response to Therapy.

The keystone in therapy, up till 10 years ago, for patients with tobacco amblyopia had been abstinence from the tobacco habit; in addition, vasodilatory drugs and strychnine had been used with doubtful success. With the growing awareness of the part played by malnutrition in this disease, preparations of vitamin B12 were used in this analysis. Of the two preparations used, hydroxocobalamin was quickly

found to be superior to cyanocobalamin and all patients ultimately came to be treated with the better drug.

The mean period that patients remained under observation was nineteen months, with a range from five months to thirty five months. The majority (40 in number) had a visual acuity of 6/12 or better after the period of follow up. Ten patients became lost from the analysis by reason of default or death. Of the fifteen patients who had a poor restoration of vision, four were undergoing their second attack of the disease.

The progress of the disease whilst on therapy was also observed by following the alteration in colour discrimination. It was found that the results fitted an exponential curve equation. By this means the rate of improvement in colour discrimination was compared in tobacco amblyopia complicated by pernicious anaemia, pre-pernicious anaemia, and diabetes. The rate of improvement in uncomplicated tobacco amblyopia treated by hydroxocobalamin was equally good as that treated by abstinence from tobacco.

Chapter VI. Leber's Hereditary Optic Atrophy and Optic Neuropathy of Pernicious Anaemia.

Leber's Hereditary Optic Atrophy is an inherited disease which primarily attacks the young adult, both eyes being affected and resulting in a serious visual defect. Abnormalities in cyanide detoxication products, similar to those found in tobacco amblyopia, were found in such patients. These changes underwent similar alteration after treatment with hydroxocobalamin, as had been demonstrated in tobacco amblyopia. The ocular features of patients suffering from the optic neuropathy of pernicious anaemia who smoked, were identical to those found in patients suffering from tobacco amblyopia. Accordingly, it is felt that the diagnosis of optic neuropathy of pernicious anaemia be reserved for those non-smoking pernicious anaemia patients who have visual signs and symptoms. Three case histories are examined which reveal the superiority of hydroxocobalamin therapy over cyanocobalamin in this condition. In one patient the visual defect commenced after treatment with cyanocobalamin for the haematological defect had been continued for some time. An occurrence not previously reported in the literature.

Chapter VII. Conclusions

In this chapter, the evidence in favour of a disturbance in the vitamin B12/cyanide relationship, as the basic factor in the production of tobacco amblyopia, is collected and examined. In the main such a disturbance leads to a failure of the detoxication of cyanide to thiocyanate, by its union with sulphur. It is difficult in the light of present knowledge to explain the rise in thiocyanate concentrations in body fluids following on hydroxocobalamin therapy, unless a hitherto unknown mechanism sited in the kidney is postulated. Such a mechanism is outlined.

CHAPTER 1.

HISTORICAL BACKGROUND

The term toxic amblyopia is generally used to designate conditions to which visual loss results from the absorption of exogenous poison or endogenously elaborated toxins. As a group these poisons have features in common. They involve the ganglion cells or optic nerve fibres of the sub chiasmal portion of the visual pathway. The visual disturbance is bilateral and, on the whole, the defect is not permanent. The common group has an affinity for the papillo macular nerve fibre bundle resulting in a central, or centro-caecal scotoma, and may be accompanied by a peripheral neuropathy. In this group are found tobacco, methyl and ethyl alcohol, lead, carbon disulphide and inorganic arsenical compounds. In the less common group, the visual defect takes the form of a peripheral contraction of the visual field while peripheral neuropathy is unusual. The quinoline group of drugs act in this way.

Tobacco amblyopia is the most common type of toxic amblyopia met with in Western Europe today. It constitutes a clinical entity which affects mainly the pipe

smoking middle-aged male, and is characterised by a bilateral impairment of central vision with the development, in the centro-caecal area of the visual field, of depressed sensitivity to red and green stimuli, without ophthalmoscopic changes. (Duke Elder 1940)

Von Graefe (1865) recognised progressive amblyopia with contracted field, and curable amblyopia with full field and central scotoma. He considered that excessive indulgence in alcohol, much smoking of strong cigars, irregular sleep and over use of the eyes might act singly, but more often acted together in producing amblyopia. Leber (1869) examined the colour sense and found a central defect. He considered that in many cases smoking was a factor in the production of central scotomata. Forster (1869) had found patients with a central scotoma who improved when tobacco smoking was avoided, and in 1871 he recorded the value of using a red object on a black background in testing for the never failing central scotoma in amblyopia from abuse of nicotine and spirits.

Although Beer (1817) is credited with the first recorded description of tobacco amblyopia, it is quite possible that Venner (1650) was referring to those cases

that are now called tobacco amblyopia, in his treatise on tobacco. Mackenzie (1830) had repeatedly hinted his suspicions that tobacco was a cause of amaurosis, and in the 4th edition of "Diseases of the Eye" (1854) he mentioned a case of amaurosis that improved on giving up tobacco without other treatment. He further considered that one of the best proofs of tobacco being a cause of amaurosis was in the great improvement of vision that ensued on giving up the use of the poison.

Lautenbach (1898) employed the term "Tobacco Amblyopia" to express retrobulbar neuritis of the optic nerve with a central colour scotoma, followed by optic nerve atrophy and occurring in those addicted to the excessive use of tobacco. Uhthoff (1886) and Groenouw (1892) regarded retrobulbar neuritis and toxic amblyopia as different diseases in spite of the seemingly identical findings. According to Uhthoff retrobulbar neuritis is distinguished from toxic amblyopia by the extent of the scotome. Early observers noted that tobacco amblyopia was almost exclusively a male disease (Nelson 1880 and Lyle 1905), and was very rare in women (Berry 1884, Eales 1887, Gunn 1887, Cossu 1923, Usher 1927a and Traquair 1928). Uhtoff (1911) warned that one should not assume a special predisposition in men, since

women were also affected when sufficiently exposed to tobacco as was found in female tobacco workers by Legge (1922).

Virtually all authorities are agreed that tobacco amblyopia is a disease of the middle-aged male beetween the ages of 40-60 years (Nelson 1880, Ramsay 1895, Lyle 1905, Bar 1906, Usher 1927_a, Traquair 1930, Lillie 1934, Greeves 1936, Hambresin and Schopens 1946 and Heaton et al 1958), being rare before the age of 30 years (Groenouw 1892) though Traquair (1930) repeated its occurrence in the 20's and Usher (1927_a) in the teens. The incidence declines after the age of 60 years (Groenouw 1892) though Traquair (1930) and Heaton et al (1958) reported its occurrence in patients of 80 years and older. Dowling (1908) extensively investigated Negro tobacco workers and concluded that this race had an immunity to tobacco amblyopia. This work has not been confirmed or disproved. Lopez (1900) stated that tobacco amblyopia was an exceptional disease in Spaniards and Cubans but this was subsequently disproved (Finlay 1901). Van den Hoeve (1927) stated that he had never seen cases of tobacco amblyopia in Holland and believed that it did not occur there, though he could offer no reason why.

Groenouw (1892) stated that all of the usual forms of using tobacco may lead to tobacco amblyopia; Lautenbach (1898) was convinced that tobacco must be smoked to produce the disorder. Doggart (1959) reported a case from the use of snuff and Chisholm (1890) from chewing tobacco. Tobacco amblyopia was common in patients who smoked tobacco as cigars or pipes, usually strong tobacco and pipe smoking was the commonest cause. (Greeves 1936, Leishman 1951 Doggart 1959). Lillie (1934) had never seen the disease from cigarette smoking, but Evans (1959), Cohen (1959), Smith (1959) and Heaton et al (1958) had. Leishman (1951) suggested that a possible explanation of the different incidence rates of tobacco amblyopia in pipe and cigarette smoking may lie in the different routes of absorption of the toxic agent. In pipe smoking the agent was swallowed causing a slow upset of gastric function with resulting metabolic derangement which in turn might produce the lesion of tobacco amblyopia.

It is agreed that prolonged exposure to tobacco is necessary to produce tobacco amblyopia (Galezowski 1883, Nettleship 1887, Chisholm 1890, Ramsay 1895, Heaton et al 1958, Doggart 1959). Chisholm (1890) had never seen tobacco amblyopia from tobacco use of less than 10 years and all but

one of the patients of Heaton et al (1958) had smoked for 30 years or more. Nettleship (1887) recorded the disease after one year's smoking. Greeves (1936) and Heaton et al (1958) found that the absolute amount of tobacco was not a determining factor, as there is no demonstrable relationship between this and the onset of the disease.

In men Berry (1887) found that one ounce to half a pound or more weekly was the quantity smoked by cases of amblyopia. The disease is seen from time to time in those who have smoked surprisingly small quantities of tobacco. Such cases have been recorded by Eales (1887), Berry (1887), Habershon (1888), de Schweinitz (1900) and others. Chisholm (1887) recorded a case that smoked only half a cigar daily. It frequently made his patient sick and he had been persevering for years to acquire the habit of smoking.

The onset of tobacco amblyopia has been described as sudden, rapid or abrupt (Hartridge 1886) or slow, gradual or insidious (Ramsay 1895, Lyle 1905, Dowling 1908, Hambresin and Schepens 1946). The earlier observers tended to emphasise the rapidity and the later observers the slowness of onset. According to some observers failure of sight in tobacco amblyopia progressed

rapidly for a time and then remained relatively stationary (Nettleship 1887) or progressive (Lyle 1905, Dowling 1908). Most authors are in agreement that tobacco amblyopia never progressed to complete loss of sight (Uhthoff 1880, Berry 1882, Traquair 1930) though Marshall and Seiler (1942) found that tobacco amblyopia accounted for 0.124% of blind registrations.

The prognosis is generally favourable if the patient gives up smoking and comes under early treatment (Ramsay 1895, Dowling 1908, Traquair 1930). Griffith (1887) from his study of cases, concluded that there was a tendency for recovery to take place even without complete discontinuance of the toxic agent. Speedy recoveries were marked in those who gave up tobacco completely. Of his 65 examples of tobacco amblyopia, 27 patients completely recovered their sight (18 complete abstinence and 9 almost complete), 24 partially recovered their sight (II complete abstinence), II remained stationary (5 complete abstinence), 3 became worse (I complete abstinence). Somewhat similar results were found by Evans (1939) - out of 55, 23 recovered fully their sight, 27 had partial recovery, 5 no recovery. Carroll (1937-44) found that patients on adequate diets made partial or complete

recovery, in spite of their continued and unabated use of tobacco and/or alcohol. He claimed his results were as good as any previous series including those in which the patients abstained from smoking. If the patient with tobacco amblyopia discontinued smoking his vision usually improved but if he continued to smoke and take large doses of vitamin B complex and a well balanced diet there would be improvement over a period of months (Carroll 1956).

The time for recovery has been variously described as rapid (Ruata 1925) or slow (Greeves 1936); not earlier than 2 months (Berry 1887), 3-42 months in 50% of patients (Griffith 1887), 2-10 months (Hambresin and Schepens 1946). Riddell (1936) stated that recovery may take up to 2 years - longer than was usually considered. Berry (1887) noticed that there was a latent period after treatment was commenced before appreciable change occurred and Traquair (1930) noticed that vision, in some cases, became worse before improvement set in, after smoking was stopped. Berry (1887) noted that relapses were rare, Gunn (1887) and Eales (1887) had not seen a recurrence though Nettleship (1887) had encountered a relapse.

According to Groenouw (1892) the typical cases

were found in middle-aged men who were heavy smokers and consumed alcohol. The general condition was disturbed with lack of appetite, insomnia, constipation and a feeling of fatigue and depression. As described by Traquair (1930) the patient was usually a man of about 50 years whose sight had been failing for several weeks or months. There was a smell of stale tobacco about him. There may be tremor of the hands. The vision failure was worse for near vision and identity of colours. The vision was better at dusk than in bright daylight. Occasionally a silvery mist surrounded any object looked at. The symptoms came on gradually and without any exciting cause. In general, there appeared to be little or no characteristic ophthalmoscopic findings, (Nettleship and Edmund 1883, Traquair 1930, Carroll 1935, Hambresin and Schepens 1946), although pallor of the temporal half or quadrant of the optic disc had been observed (Groenouw 1892, Traquair 1930, Carroll 1935). Persistent and marked miosis had been noticed (Galezowski 1883) and had been used to differentiate tobacco amblyopia from alcoholic amblyopia in which the pupils were said to be dilated B.M.J. 1,744 (1879)

Diminution of visual acuity was one of the characteristic signs of tobacco amblyopia (Galezowski 1883). There was conspicuous disproportion between distant vision

which was almost normal in most cases, and close vision which showed pronounced deterioration (Traquair 1930). As long as the scotoma had not attacked the fixation point, the vision remained good in tobacco amblyopia. When the fixation point was affected, the decrease in the visual acuity could be rapid (Hambresin and Schepens 1946).

The visual field defect has been variously described as central (Leber 1869, Galezowski 1883, Connor 1890, Hedges 1957) and centro-caecal (Traquair 1930, Carroll 1935, Heaton et al 1950) in which the defect for colour was larger than that for white, and of the colours that for red and green being larger than that for blue (Lyle 1905). Heaton et al (1950) crystallised the literature in the following criteria:-

(1) The patient must be a smoker.

(2) A centro-caecal scotoma must be present.

(3) This scotoma must be horizontally oval and most readily detected by a reduced stimulus.

(4) The defect for colour must be larger than that for white.

(5) The scotoma must be bilateral though not necessarily equal on the two sides.

Pathological changes in tobacco amblyopia or

tobacco-alcohol amblyopia had been thought to involve either the circulatory or the neural elements of the eye, or both, although in actual examination, it was exceptional to find such changes (Lyle 1947). Histopathologic findings have been reported in detail (Samelsohn 1882, Sachs 1887-93, Birch-Hirschfeld 1902, Victor and Dryfus 1965). Wordworth (1863) stated that only one pathologic condition was seen - namely white atrophy of the optic nerves. Sachs (1887) considered that the generally valid anatomic basis of tobacco amblyopia was the partial degeneration of the optic nerve tract and degeneration of the papillo-macular fasciculus. Nuel (1896) claimed that the central scotoma of tobacco amblyopia was the result of macular disease and not an interstitial neuritis of the optic nerve. Lillie (1934) considered the idea that only the maculopapillary bundle of nerves is affected seemed best established clinically. Groenouw (1892) favoured the view that the primary site of tobacco amblyopia was to be found in the optic nerve and not in the chiasma or optic tract. Lyle (1905) believed that the amblyopia was centred on a primary degeneration of the ganglion cells of the retina in the neighbourhood of the macula lutea with a secondary degeneration of the nerve fibres arising from the cells. Victor and Dryfus (1965) took the opposite view. The interstitial changes noted were considered to be an

accompaniment of the degenerative process in the optic nerve itself. Schieck (1903) and Bar (1906) supported the view of Parsons (1901) that the action of nicotine in tobacco amblyopia was in part vascular, causing vasoconstriction of the arterioles, which explained the selection of the macular region with its unique vascular supply. Schieck (1903) felt that the nerve fibres which maintain the retinal centre were unfavourably situated in the axial part of the optic nerve and therefore were liable to reflect nutritional disturbances, earlier than the fibres located in the periphery of nerve. Thus, tobacco was said to act on the nerve by way of a chronic nutritional disturbance. Several authors had noted that the vision in tobacco amblyopia was improved by vasodilators and had considered that this supported the hypothesis that tobacco amblyopia was due to vascular spasm in the visual pathway (Cordes and Harrington 1935, Duggan 1935-37). Carroll (1937) challenged this as he had no success with sodium nitrate. It would appear that the lesion in tobacco amblyopia was primarily nervous rather than vascular (Gunn 1930) and although the factor of vaso spasm could not be excluded, it is probably not of very great importance (Evans 1939). Schepens (1946) suggested that tobacco amblyopia began with an enlargement of normal

angioscotomata particularly in the centro-caecal regions. Neuschueler (1928) summed up the theories proposed to explain tobacco amblyopia:-

(1) Primary interstitial inflammation of the papillo-macular bundle with predominating localisations in the optic canal and subsequent compression of nerve fibres by the newly formed tissue (Uhthoff 1911).

(2) Primary lesion in the vasal system consisting of inflammation and thickening of the walls, often the phenomenon of endararitis (Schieck 1903).

(3) Primary degeneration of the nerve fibres of the papillo-macular bundle with secondary and simultaneou lesions of the ganglion cells of the macular region (Dalen 1906).

(4) A primary lesion of the centre of the retina with secondary ascending degeneration of the papillo-macular bundle (Roenne 1910).

He further concluded that none was satisfactory.

Parsons (1901) considered that the action of nicotine (or, rather of the unknown cause of tobacco amblyopia) was two-fold; (1) vascular, causing vaso-constriction of the arterioles; (2) paralytic upon the synapsis either of the

cone fibres, or of the cone bipolars, or of both, and Fisher (1901) felt that nicotine was directly toxic to the ganglion cells of the retina. Ramsay (1895) wrote "as far as my own observations go, all cases of tobacco amblyopia when recovery is incomplete will sooner or later exhibit peripheral contraction of the visual field." These observations suggested that parts of the retina and optic nerve other than those connected with the papillo-macular bundle of nerve fibres were involved, though in some cases the nerve fibres even in the papillo-macular bundle were irregularly affected. Doyne (1889) suggested that tobacco might have a toxic influence on a hypothetical substance in the retina analogous to the visual purple, degenerating it and causing retinal exhaustion, which shows itself in the failure of the more delicate colour sense. The exhaustion naturally takes place at the point of greatest retinal activity and where the light is proportionately stronger, the rays being more accurately focussed. Schanz (1920) held the opinion that in toxic amblyopia the retina was damaged by light while the poisonous substances acted as sensitising agents.

Recent experimental and clinical evidence both suggest that there is some connection between the metabolism of Vitamin B12 and that of cyanide, and that smoking, which is

associated with a high cyanide intake (Surgeon General U.S. 1964, Darby and Wilson 1967) may adversely affect Vitamin B12 metabolism (Boxer and Rickards 1952, Wokes and Piccard 1955, Braekkan et al 1957, Wokes 1958, Smith 1961, Smith et al 1963, Smith 1964, Smith and Duckett 1965, Matthews et al 1965, Wilson and Matthews 1966, Lindstrand et al 1966, Smith and Foulkes 1966). The hypothesis that interconnected disturbances of cyanide/Vitamin B12 metabolism may be concerned in the pathogenesis of tobacco amblyopia, the retrobulbar neuritis of pernicious anaemia, Leber's hereditary optic atrophy, and certain tropical neurological syndromes apparently associated with a high cyanide intake from tropical pulses such as cassava, is supported by Smith (1961), Wilson (1965_a), Montgomery (1965), Wilson and Langman (1966), Monekosso and Wilson (1966), Freeman (1967), Chisholm et al (1967), Foulds et al (1968 a, b, and c), Linnell et al (1968), and Osuntokun et al (1969).

CHAPTER II.

OPHTHALMOLOGICAL INVESTIGATION.

The material for this manuscript was obtained from the out-patient and in-patient investigations carried out on 65 patients suffering from tobacco amblyopia, collected over a period of 3 years. The patients were referred from Ophthalmological clinics in the area of the Western Regional Hospital Board of Scotland, principally from Glasgow and its immediate surroundings.

Using the criteria of Heaton et al (1958) as a guide the diagnosis was confirmed on the finding of bilateral depression of vision, an acquired defect of colour vision, and centro-caecal defects in the field of vision, occurring in a smoking subject.

The patient age ranged from 46-84 years, all but one were male and their tobacco consumption lay in the range 0.5-7 ozs. per week. The patients were informed that it was not necessary to alter their smoking habit but five patients elected to abstain from smoking and this was their only treatment.

In general, the patients sought medical advice

after their visual symptoms had been present for 6 months or so, by which time the visual loss of both eyes was substantial. A number of the patients were ill due to coincident disease and this was categorised after investigation. In a few the coincident disease was the reason for seeking medical advice and they had the visual complaint subsequently categorised.

Information about a patient's direct vision can be obtained by examining his form sense or visual acuity, his colour sense and his light sense. By examining his field of vision information is gained about his indirect vision. The patients in this analysis of tobacco amblyopia had assessments of their visual acuity, colour vision and fields of vision carried out to confirm the diagnosis and at intervals whilst on treatment.

Visual Acuity

As the disease process does not affect the eyes equally, the visual acuity is not equally depressed in tobacco amblyopia. According to the majority of observers, vision is poor in sunshine, but improves in the evening or in subdued lighting. (Nelson 1880, Groenouw 1892,

Harman 1904, Traquair 1930, Carroll 1935). This is due to the glimmering mist which covers all objects, being removed with twilight and allowing contours to become sharper. Hirschler (1871) claimed that this was so only for large objects and did not apply to reading.

The distance visual acuity was estimated subjectively by means of Snellen's test type at 6 metres (Snellen 1862). In each case the visual acuity recorded was the best visual acuity with a spectacle correction.

The near vision was similarly estimated using the notation laid down by the Faculty of Ophthalmologists (Law 1951-52). Examination of the best distance visual acuity obtained from the 65 pairs of eyes, showed that in only 22 was the visual acuity of the right equal to that of the left, in 18 the visual acuity of the right was better than that of the left, and in 25 it was worse. Thus confirming the unequal visual loss in this disease. As four of the left eyes were amblyopic from other causes (3 from long standing squint, 1 from a central retinal artery occlusion) and, as no improvement in vision could be expected in them, the right eye of all patients was selected for all visual comparisons.

The visual acuity (Snellen) was converted to percentage visual acuity (Ridley 1959). The visual acuity in untreated tobacco amblyopia was found to be within the range 1.5 - 100% with a mean at 18% (equivalent to a visual acuity of 6/36 Snellen). Of the 65 patients 21 had a visual acuity of 6/24 or better and 44 a visual acuity of 6/36 or worse. The improvement in visual acuity with therapy is dealt with in the section on treatment.

The near vision results were similarly transposed into the percentage visual acuity scale. The range for the right eye was 8% - 64% with a mean at 20.75% which is equivalent to N18 at a standard reading distance of 15 inches or 38 cms. The disproportion alluded to by classical writers between distance and near vision was not confirmed ($t = 0.57$; $n = 116$; $p > 0.1$).

The Colour Sense

The colour sense can be tested by various methods some of which give only a rough estimate, while others are a very sensitive index. The methods are as follows:-

(a) Colour Naming

By this means a subject's ability to name correctly

or incorrectly test colours will reveal gross defects of colour vision. Inability to distinguish one primary colour from another is termed gross colour confusion.

(b) Pseudo-isochromatic Plates or Confusion Charts.

These plates made up of coloured dots in which the background colour differs from that of the figure (be it a number or symbol). These plates have been deliberately constructed so that the background and figure colours lie at different points on a known confusion axis for one of the congenital dichromats. See Fig 2, 1. By presenting a series of appropriate plates, a patient can be quickly screened for the four congenital colour deficiencies. The plates that are available are after the pattern of Stilling (1883), Dvorine (1953), Hardy-Rand-Rittler (1955), Ishihara (1959), and the Tokyo Medical College (1957).

(c) Pigment Matching Tests.

These test a subject's ability to discriminate between colours which differ only by a small amount when viewed under a constant illumination. The Farnsworth-Munsell 100 Hue test (Farnsworth 1943) is a refined and useful member of this group. The test enables a qualitative as well as a quantitative estimation of the colour defect to be carried out (Crone 1961).

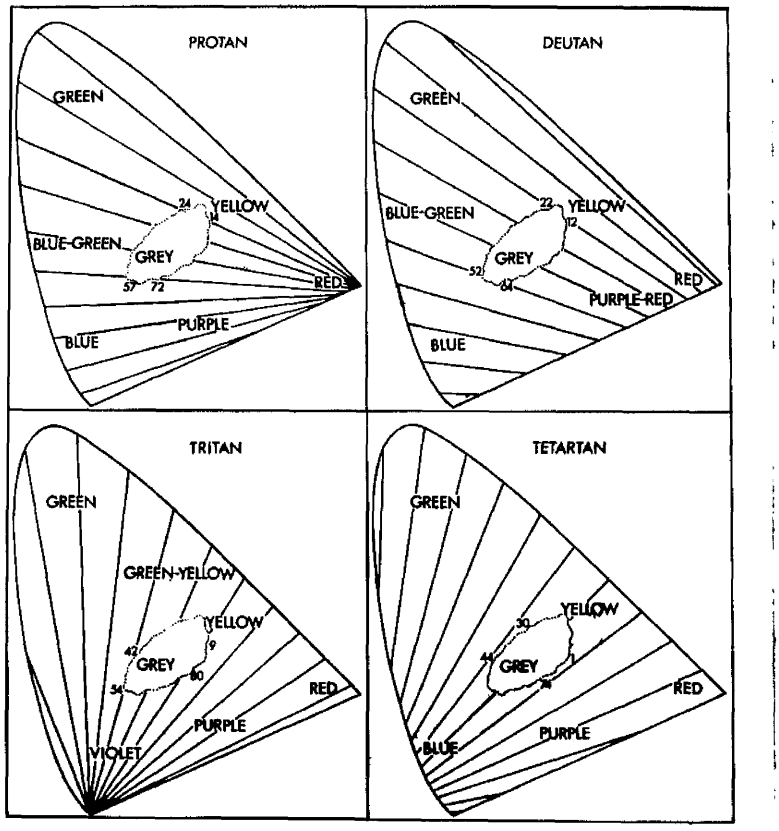


Figure 2. 1. C.I.E. Diagrams showing isochromatic lines for the known congenital colour defects. In the central area is shown the loci for the F.M. 100 Hue caps and the numbers refer to cap numbers. (After Lakowski 1968).

(d) Spectral Matching Tests.

These tests are carried out on an anomaloscope after the pattern used by Nagel (1907), or Pickford and Lakowski (1960). The subject views a halved aperture, one half containing the test spectral colour, the other is controlled by the testee. He is required to make a colour match by varying the proportions of two other spectral colours which can balance the test colour at known proportions. The Nagel anomaloscope tests for red-green colour defects (i.e. Protan and Deutan), and the Pickford-Nicolson anomaloscope for red-green, yellow-blue (Tetartan) and green-blue (Tritan) defects. This is a delicate method of assessment which requires considerable experience in its use before the interpretation of the results are meaningful.

Defective colour vision is acquired by patients born with a potentially normal colour vision system which has failed to reach maturity or has deteriorated after reaching maturity, because of local ocular disease, systemic disease or the toxic effect of systemically administered drugs. The extent of the acquired colour defect is unequally distributed between the eyes and each eye must therefore be tested separately. A typical

dyschromatopsia develops from normal trichromatic vision though an abnormal trichromatic stage before dichromatic vision is reached; further extension of the process will lead to monochromatism and eventual blindness. Pseudo-isochromatic plates are of little value in detecting the relatively early trichromatic stage which can be detected by the Farnsworth-Munsell 100 Hue test or by anomaloscope tests.

Disease processes affecting the neuro-sensory or conductive layers of the retina will if severe enough lead to blindness. Less severe disease will degrade visual function in a variety of ways including the development of an acquired dyschromatopsia. Thus lesions of the neuro-sensory retina by and large, result in a loss of colour discrimination in the yellow-blue, or violet blue-green, and lesions of the conductive layers in the red-green areas of the spectrum respectively. (Koellner 1912, Cox 1960, Verriest 1963).

The Colour Sense in Tobacco Amblyopia

One of the diagnostic criteria of this condition is the finding, in the centro-caecal area of the field of

vision, of depressed sensitivity to red and green stimuli. Accompanying this is a subjective disturbance of colour discrimination which traditionally reveals itself by the confusion of gold and silver coins, or today, between copper and cupro-nickel. Galezowski (1883) was first to draw attention to the subjective colour defect in tobacco amblyopia and Groenouw (1892) pointed out that this acquired dyschromatopsia differed from that of the congenital dichromat. In more recent times Riddell (1936) observed that with treatment the colour sense in tobacco amblyopia took much longer to return to normal levels, if at all, than did visual acuity. Cox (1960), Francois and Verriest (1961) Saraux et al (1966), Bouniq and Cosces (1966), observed that the dyschromatopsia found in tobacco amblyopia had features in common with other toxic amblyopias.

Of the tests available for investigating the colour sense, the Farnsworth Munsell 100 Hue Test was found the most useful in the investigation of the tobacco amblyopia patients. The test consists of a graded series of 85 coloured caps arranged in four boxes. The patient is required to arrange the colour caps into a regular colour series between fixed end caps. He is presented with the

coloured caps arranged in a standard random fashion. Each cap is numbered on its reverse side. The patient's arrangement of the caps is recorded on a chart, (Fig 2, 2), and deviations from the normal arrangement are soon apparent. As the caps are numbered, the error of any particular cap is obtained by summing the differences between the cap number of the cap in question and the cap number which comes before, and the cap which comes after it. If the patient's arrangement is normal the minimal error score for any cap is 2. Two is accordingly deducted from individual cap scores and the sum of these individual error scores gives the total raw score. The individual error score of the caps may also be expressed graphically as the patients profile. (Fig.2, 2).

This test was originally designed as a binocular test, for the screening of youthful subjects for congenital colour defect, and a time limit of 2 minutes was placed for the completion of each box. This routine required amendment for the investigation of the dyschromatopsia of tobacco amblyopia. Each eye was examined separately, and no time limit was set for the completion of each box as the patients being tested were elderly, many had defective

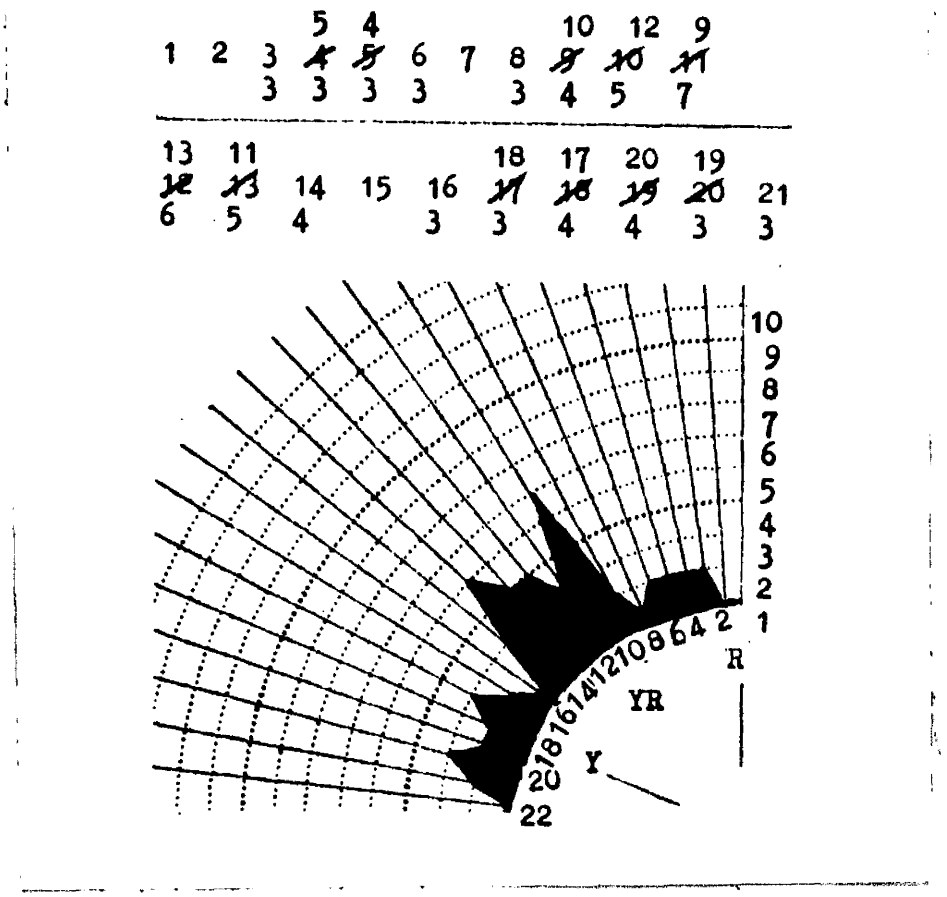


Figure 2. 2. Showing specimen Farnsworth Munsell 100 Hue calculation and profile.

vision and many had difficulty in manipulating the caps. Standard artificial illumination was provided by a Hubble Veri Vidi cabinet which provided artificial daylight illumination with a colour temperature 6,500 K with an intensity of 1200 -1290 lux at the test caps, thus conforming with B.S. 950 part one (1967).

The materials used in this test come from all parts of the colour circle and hue discrimination near the centre of the colour space can be tested in all directions. One of the great merits of the Farnsworth Munsell 100 Hue test is that elements suitable for detecting colour confusion can also be used for detecting the variations in colour discrimination existing among trichromatic observers. (Fig. 2, 1). To be able to measure these variations tasks presented for discrimination must include small colour differences ($\triangle$ E) such as are found in this test, where $\triangle$ E between successive caps is of the order 2.5 N.B.S. units (Nation Bureau of Standards). The task here may be considered analogous to visual acuity testing (Lakowski 1968). Subjects with acute colour discrimination will arrange the "colour series" in each box within the two end limits correctly, those with lesser discrimination

will accumulate "error scores" which are a measure of the degree of displacement from the ideal arrangement.

Verriest (1963) demonstrated that in normal healthy subjects the error score of the Farnsworth Munsell 100 Hue test increased in a positive manner with age, after 20 years of age. (Fig. 2, 3). This was in agreement with the earlier finding of Lakowski (1958) who described distinct phases in the normal development of colour vision. Colours are perceived and discriminated most accurately between the ages of 16 and 35 years. After 55 years there is a rapid deterioration in ability for fine colour discrimination. Red-green discrimination is least affected by age, but yellow-blue and violet blue-green discrimination may deteriorate from as early as the 30th year. Lakowski (1962) produced evidence that these observed deteriorations in colour discrimination were due to retinal rather than pre-retinal changes.

Each patient performed the Farnsworth Munsell 100 Hue test on more than one occasion prior to the commencement of treatment. The error score for the right eye of each patient is presented in this analysis. For the 65 patients, prior to treatment the error score lay in

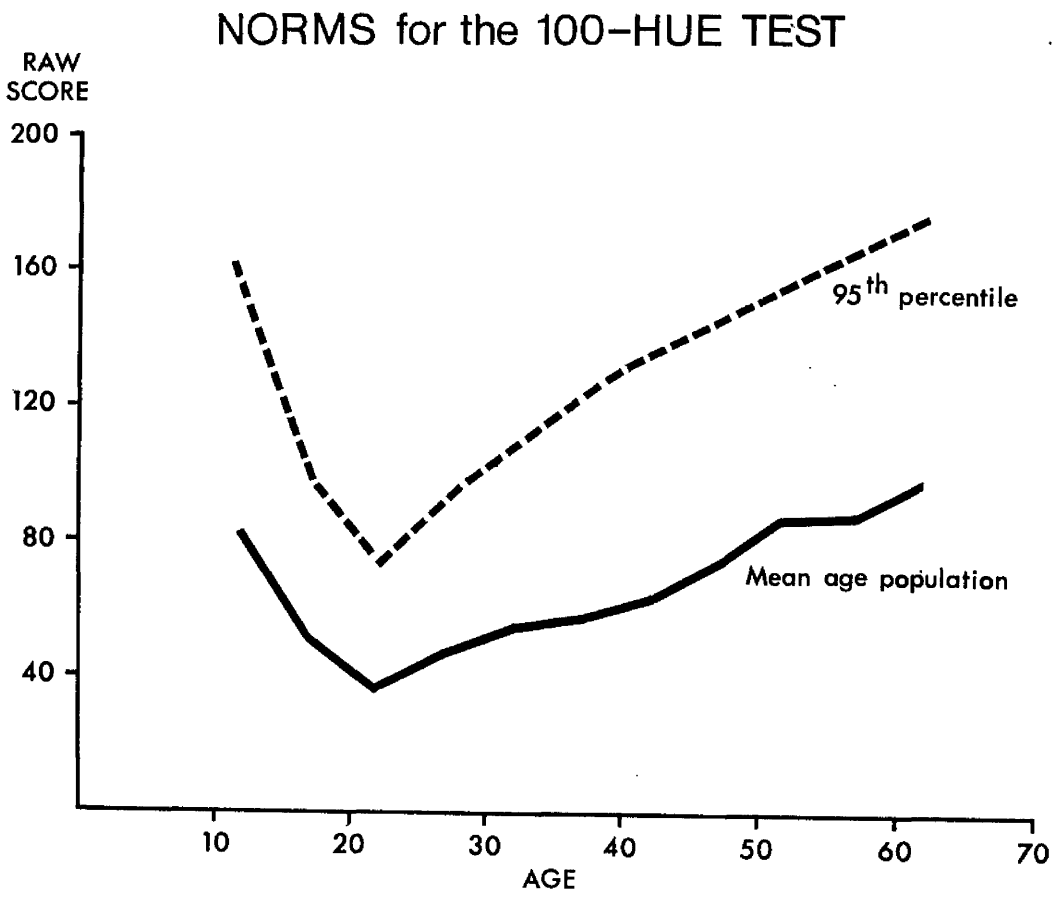


Figure 2, 3. Mean Farnsworth Munsell 100 Hue test error score in normal patients related to age, (after Verriest 1964) and showing the upper 95th per cent limit.

the range 260-1290 with a mean at 732± 236. As a group these error scores are much higher than previously recorded analyses - Verriest (1963) on normals, Kinnear (1965) on diabetics, Lakowski and Davenport (1968) on chloroquine treated arthritics: and some doubt must be cast on the validity of scores above 1000 which can be achieved by randomizing the caps. A typical Farnsworth Munsell 100 Hue profile is shown in Fig. 2, 4.

In order to investigate the validity of the test at high error scores an investigation was carried out into the change in error score on two tests and on multiple testing.

(1) Comparison of the difference between 1st and 2nd test and initial error score.

83 patients were selected at random and tested. These patients included examples of tobacco amblyopia, Leber's hereditary optic atrophy, macular degeneration, squint, glaucoma and endocrine disease. One eye at random from each patient was retested after 24 hours.

The relevant figures are contained in table 2, 1

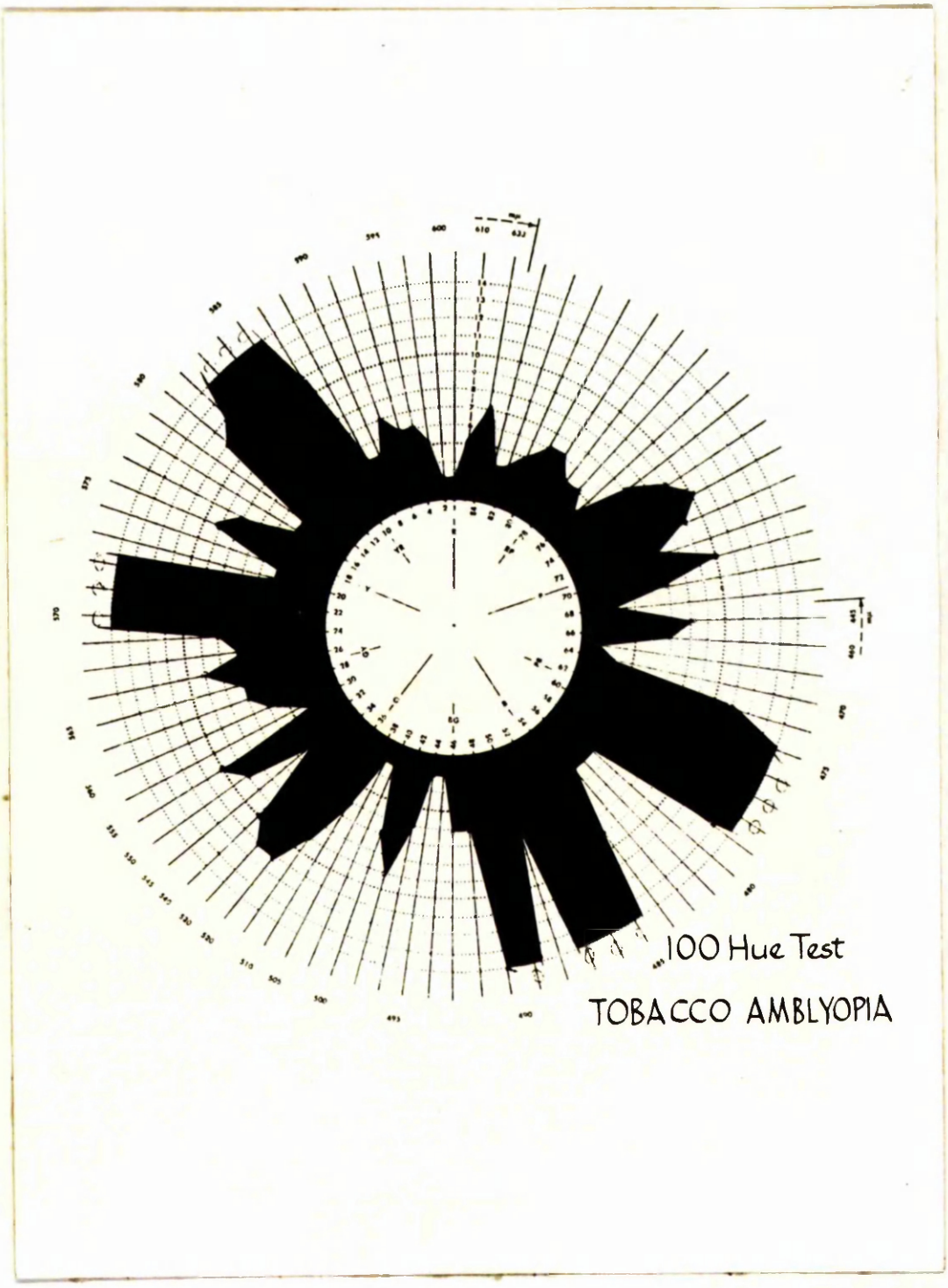


Figure 2, 4. Anarchic profile in Tobacco Amblyopia, but with preponderance of error in the red-green areas.

TABLE 2,1.

CHANGE IN 100 HUE ERROR SCORE ON TWO TESTS WITH INITIAL ERROR SCORE

GROUP	0 - 200	201 - 400	401 - 600	601 - 800	801+
NUMBER IN GROUP	18	19	14	18	14
MEAN CHANGE	- 15	+ 0.31	- 9.4	- 9	- 53
STANDARD DEVIATION	± 49	± 56	± 76.5	± 141	± 166
95 PERCENTAGE	± 96.04	± 109.76	± 149.94	± 276.36	± 325.36

and figure 2, 5. As can be seen in Figure 2, 5, at error scores above 601 there appears to be a trend towards spontaneous improvement on retesting. However, the mean change in error score in this group as in each of the other groups is not significantly different from the expected value of zero (601-800; $t = 0.27$, $n = 17$, $p > 0.1$; 800+; $t = 1.19$, $n = 13$, $p > 0.1$).

With increasing initial error score however, there is an increased scatter of results, the increase in variance being statistically significant only for tests at error scores of over 600. (601-800; $F = 2.87$, $p < 0.05$; 801+, $F = 3.38$, $p < 0.05$). There is no evidence to support the premise that repetition of the test improves performance.

From these figures one can deduce by how much the error score at each particular level must change to be regarded as a significant alteration in the result of the test. This is illustrated in Figure 2, 6. Here the 95% range about the mean is shown. A change in error score lying outside these limits would have a one in twenty probability of arising by chance and would therefore be significant. For example, with an initial error score of 150, on retest a score of 39 would be a significant

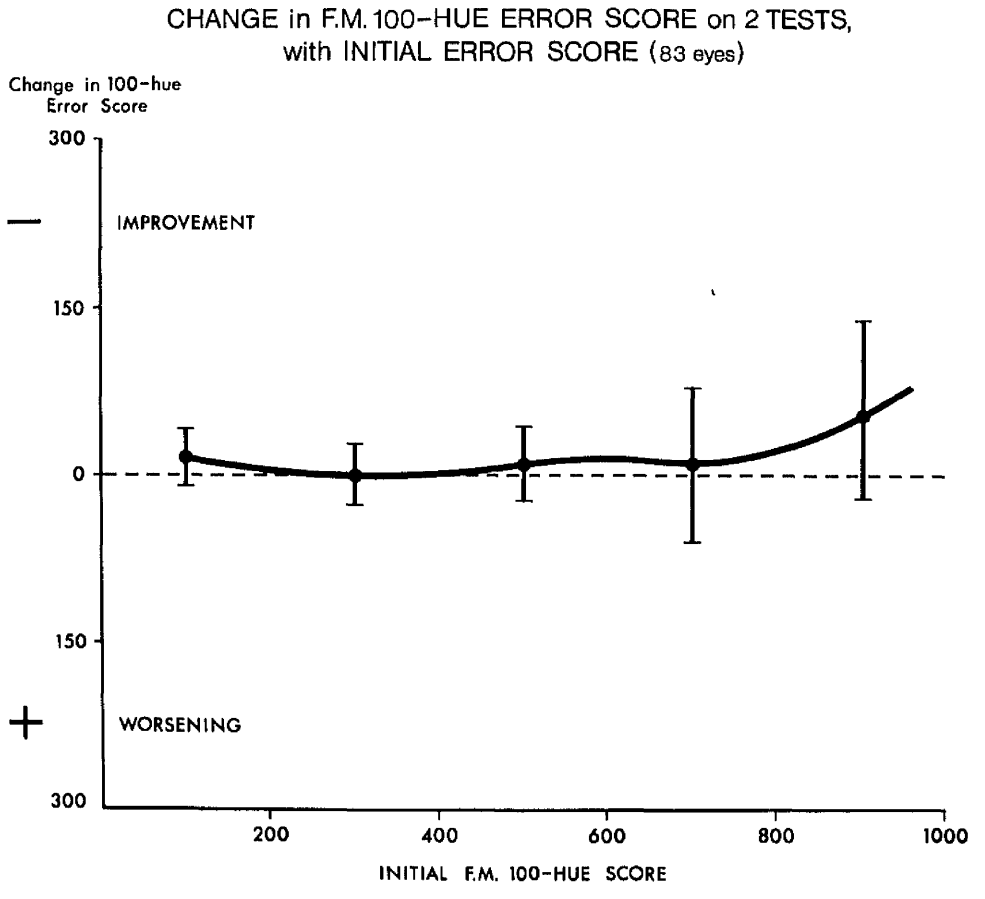


Figure 2, 5. Mean change in the Farnsworth Munsell 100 Hue test error score on 2 tests with an indication of variance compared with initial error score.

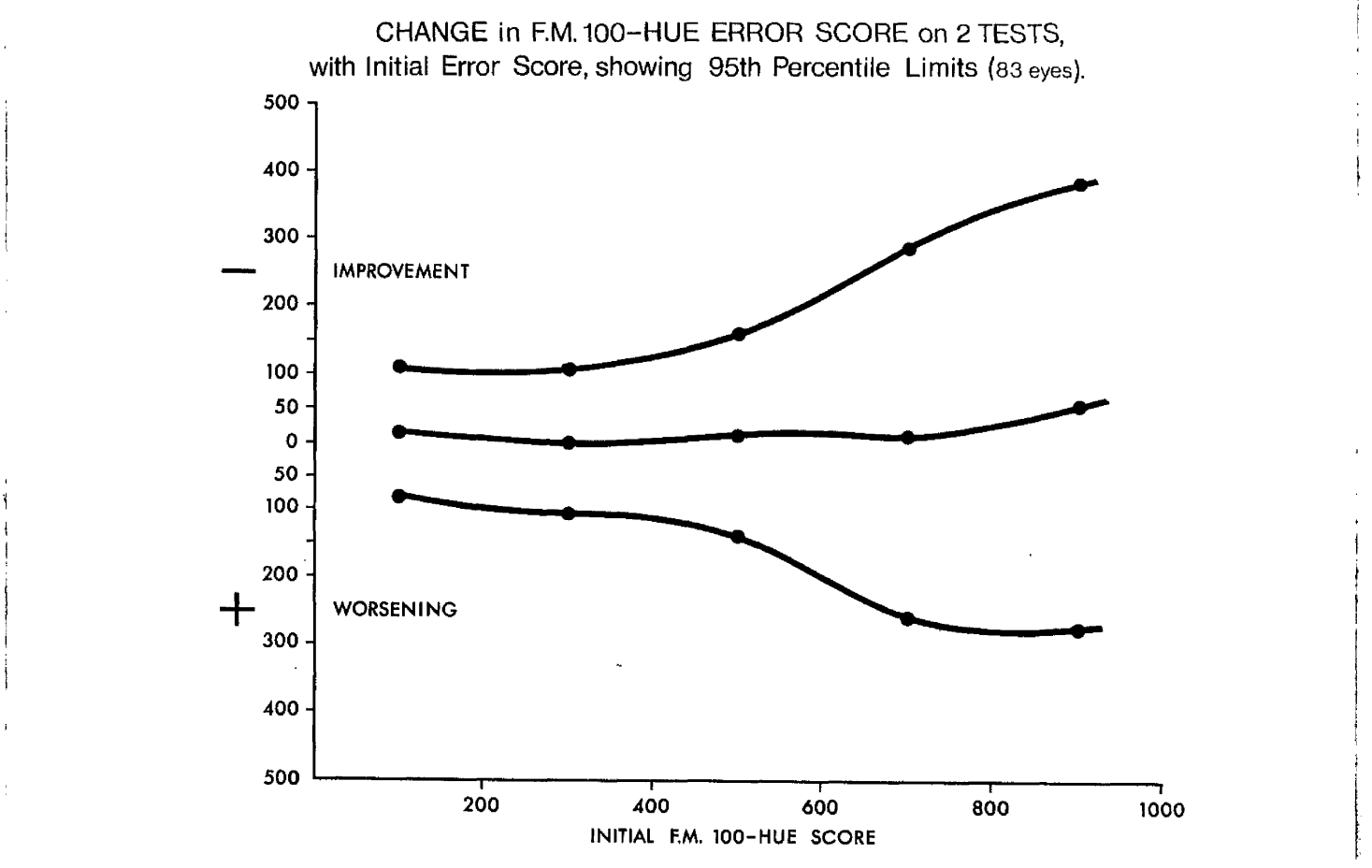


Figure 2, 6. Comparison of change in the Farnsworth Munsell 100 Hue test on two tests, with initial error score, and showing the 95 per cent limits of the variance.

improvement, or a score of 231, a significant deterioration; similarly at an initial error score of 700, a fall to 415, or an increase to 967 would be significant.

A comparison between age and change in error score and between age and error score variance for this group of patients showed no significant relationships in either case (Table 2, 2, and Figure 2, 7).

Table 2,2.

CHANGE IN 100 HUE SCORE ON TWO TESTS WITH AGE

AGE GROUP (YEARS)	30	31-45	46-60	61-75	76+
NUMBER	10	11	14	30	18
MEAN CHANGE IN ERROR SCORE	- 7.5	+ 9.45	- 72	- 35.6	+ 38.05
STANDARD DEVIATION	± 78.96	± 75.4	± 142.6	± 260.8	± 108.2

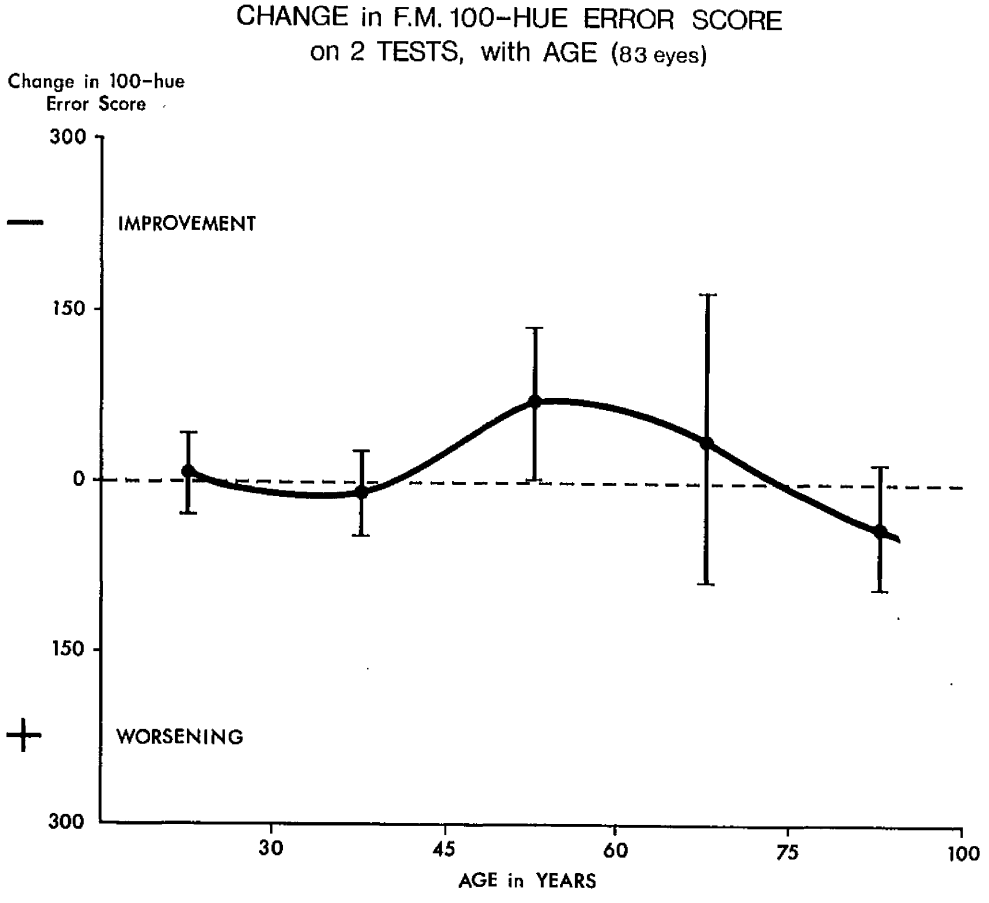


Figure 2. 7. Comparison of change in the Farnsworth Munsell Hundred Hue test score in two tests, with age. The mean of change and variance are shown.

(2) Multiple Testing.

Table 2,3.

RESULTS OF MULTIPLE TESTING

PATIENT	1	2	3	4	5	6	7	8	9
NUMBER OF TESTS	6	9	8	10	10	10	10	6	10
AVERAGE SCORE	43.6	157.44	184.75	274.8	520.5	602.7	686.5	696.3	785
STANDARD DEVIATION ±	11.76	18.25	29.5	42.6	47.45	59.2	60.14	54.6	95

The variance about the means in figure 2, 5 is made up of 2 components - the inter-observer error and the intra-observer error. To establish whether "familiarity" with the test had any bearing on the test result, patients from each group except those with error scores over 800 were selected for intensive testing over a space of 2-3 days.

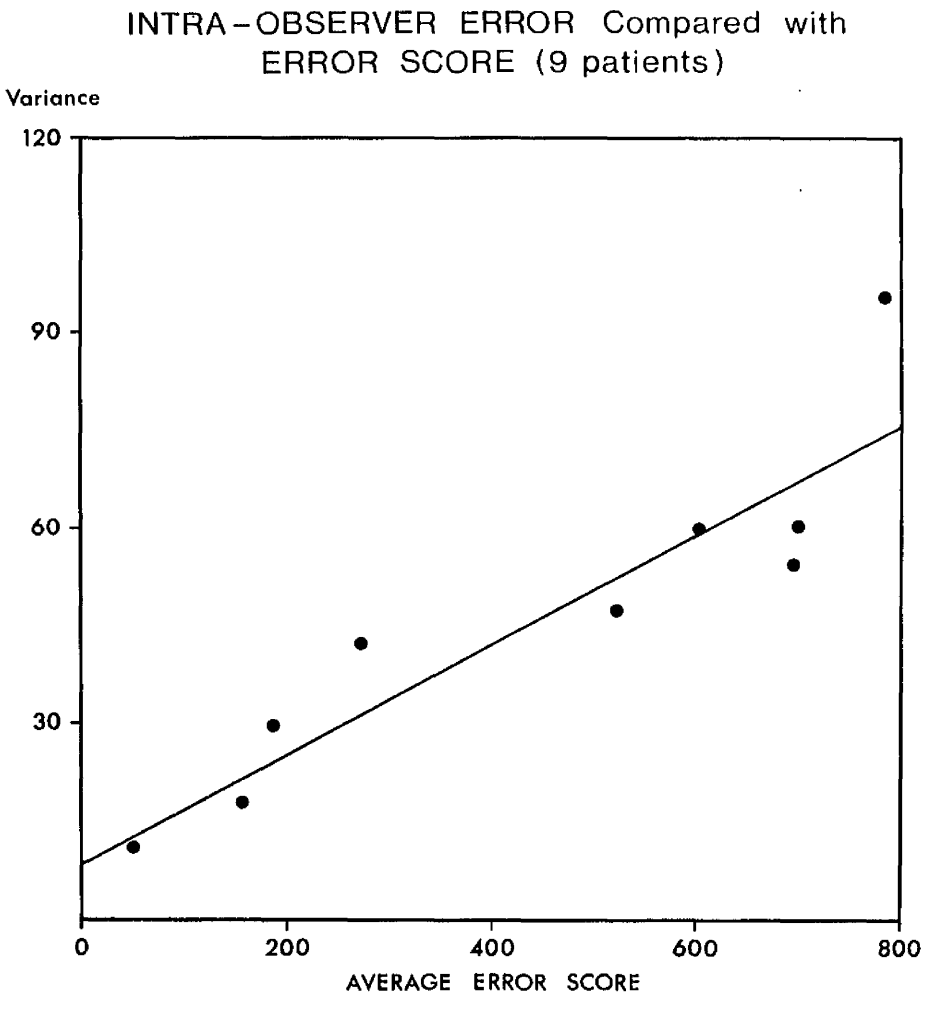


Figure 2. 6. Intra - observational error for the Farnsworth Munsell 100 Hue test. ($r = 0.91$; $n = 7$; $p < 0.001$).

In these tests the same eye was examined on each occasion. For this analysis the scatter of the results about the mean error score was used. The results are shown in table 2, 3. In no case was there any indication that repetition influenced the patient's performance.

A comparison of the average error score with the error score variance for each patient, shows a significant positive relationship ($r = 0.91$, $n = 7$, $p < 0.001$). (Figure 2, 8). As this variance is a measure of the intra observer error, it can be seen that this quantity increases with error score. The difference between this variance and the variance found on two tests is a measure of the inter observer error, i.e. error inherent in the test. This also increases with error score.

1. Comparison of F.M. 100 Hue error score and Age in untreated Tobacco Amblyopia.

This comparison was possible in 64 patients, 1 patient in the series had abstained from smoking prior to the first outpatient consultation and was therefore excluded. A positive correlation between patient age and the Farnsworth

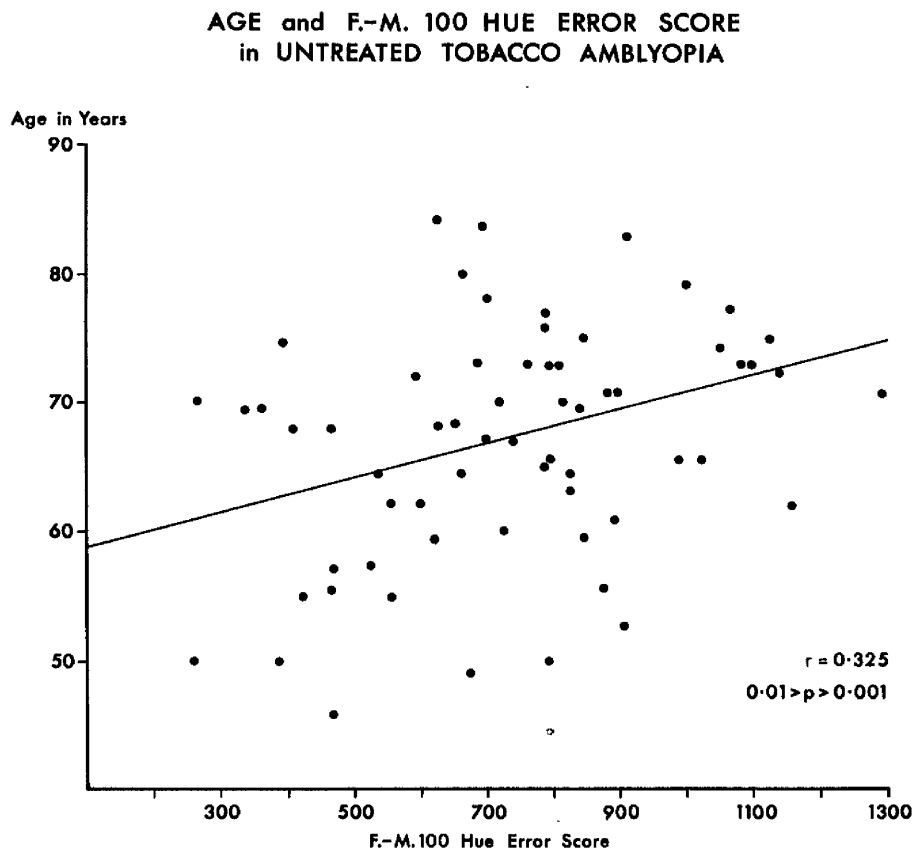


Figure 2. 9. Comparison between age in years and the Farnsworth Munsell 100 Hue test error score in untreated tobacco amblyopia. ($r = 0.325$; $n = 62$; $0.001 < p < 0.01$).

Munsell Hundred Hue test result was obtained in untreated tobacco amblyopia which was significant ($r = 0.325$; $n = 62$; $p = < 0.01$). (Figure 2, 9).

2. Comparison of F.M. 100 Hue error score and visual acuity in untreated Tobacco Amblyopia.

This comparison was possible in 64 patients. A positive correlation was obtained which was significant ($r = 0.402$; $n = 62$; $p = < 0.001$). The visual acuity - in this comparison was expressed as a percentage taking 6/6 Snellen as 100% after Ridley (1959) (Figure 2, 10).

3. Comparison of F.M. 100 Hue error score and duration of symptoms in untreated Tobacco Amblyopia.

The comparison was carried out on 51 patients whose duration of symptoms was definite. A positive correlation between these 2 factors was obtained which was just significant. ($r = 0.242$; $n = 49$; $0.1 > p > 0.05$). Thus patients with a long history of visual disturbance tended to give a poor result on colour vision testing.

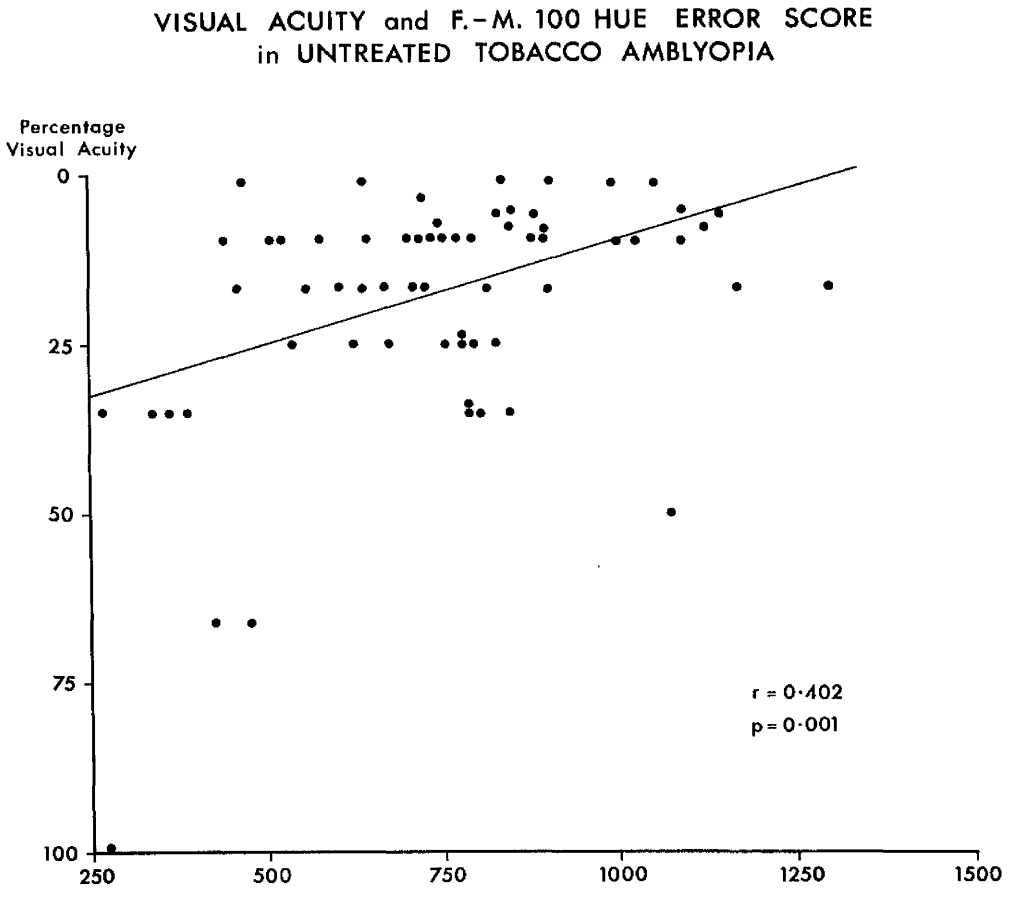


Figure 2. 10. Comparison between Percentage visual acuity and Farnsworth Munsell 100 Hue test error score in untreated tobacco amblyopia. ($r = 0.402$; $n = 62$; $p < 0.001$).

4. Comparison of F.M. 100 Hue error score and serum vitamin B12 concentration in untreated tobacco amblyopia.

This comparison was carried out on 60 patients. A positive correlation was obtained but this proved not to be significant. ($r = 0.161$; $n = 58$; $p > 0.1$).

5. Comparison of F.M. 100 Hue error score and serum Folate concentration in untreated tobacco amblyopia.

This comparison was carried out on 51 patients. A positive correlation was obtained which proved not to be significant. ($r = 0.166$; $n = 49$; $p > 0.1$).

The Field of Vision.

The investigation of a patient's indirect vision is carried out by examining his field of vision. This can be accomplished by several means:-

1. Kinetic Examination of Field of Vision.

(a) The confrontation test is simple and easily applied. In

its execution the observer tests the range of the patient's field of vision by that of his own. The observer and patient sit opposite each other at about one metre apart, a hand or test object is introduced in the mid plane between opposite eyes. When the test object comes into view the position is noted. The test can be repeated in as many meridians as necessary. It is of value in examining the field of vision in children.

(b) A well established method of delineating the field of vision accurately is by perimetry. The perimeter consists of a half sphere, or rotatable arc revolving around a pivot in order to test various meridians. The arc of the circle is approximately concentric with the retina. The patient with one eye occluded, fixes a stationary central mark at a distance of $\frac{1}{3}$ of a metre. The test object is moved in from the periphery until it is seen. By using test objects of variable size and colour, but of fixed luminance, and with a constant background illumination, the boundaries of those areas of the retina just sensitive to the given threshold are determined. The lines joining points of equal sensitivity are called isopters. (Groenouw 1893).

(c) By using a somewhat similar technique the central area

of the visual field (20° around fixation point) can be examined on the tangent screen by campimetry. The patient sits from the screen at one metre or two metres distance. The screen over which the test objects are moved is flat, and as the mode of projection is tangential, a certain amount of magnification of the defects occurs which is not so in perimetry. The central, centro-caecal and blind spot areas can be investigated very accurately by this means.

II. Quantitative Perimetry

Quantitative Perimetry (Bajx 1940, Harms 1952) or light sense perimetry (Sloan 1939) or static perimetry (Frankenhauser and Schmidt 1950) is carried out by measuring the variation of luminance of the test object to produce recognition at a series of fixed locations in the field of vision. The entire visual field can be explored from the centre to periphery in a number of different meridians, and by using a series of test objects of different size one can accurately measure both the density and extent of a scotoma.

The apparatus consists of a hemi-spherical bowl. The patient's eye is located 33 centimetres from this sphere. The

background illumination of the bowl is variable, and generally an illumination of 10asb is used (1 asb = 100 u lux). By means of neutral filters the illumination of the test object can be reduced by 80 equidistant logarithmic stages from 1000asb to 0.00001asb. The light threshold is measured at 2^{o} intervals along any given meridian and gives sections across the "island" of Traquair (1930). (Figure 2, 11).

The Field of Vision in Tobacco Amblyopia.

Though a central scotoma for colours (Red and Green) was found in tobacco amblyopia and tobacco-alcohol amblyopia by Leber (1869), Galezowski (1883) and Connor (1890), most authors have described the typical scotoma as para-central (Groenouw 1893, Lyle 1905, Doyne 1922 and others). Traquair (1930) emphasised that the scotoma was in fact centro-caecal and not central. The scotoma developed by extension from the nasal side of the blind spot and was uniform in character. It was ovoid in shape and contained nuclei of maximum density (Hambresin and Schepens 1946 and Heaton et al 1958).

Lyle (1905) showed that central vision for green was affected first, red later and blue last. Carroll (1935)

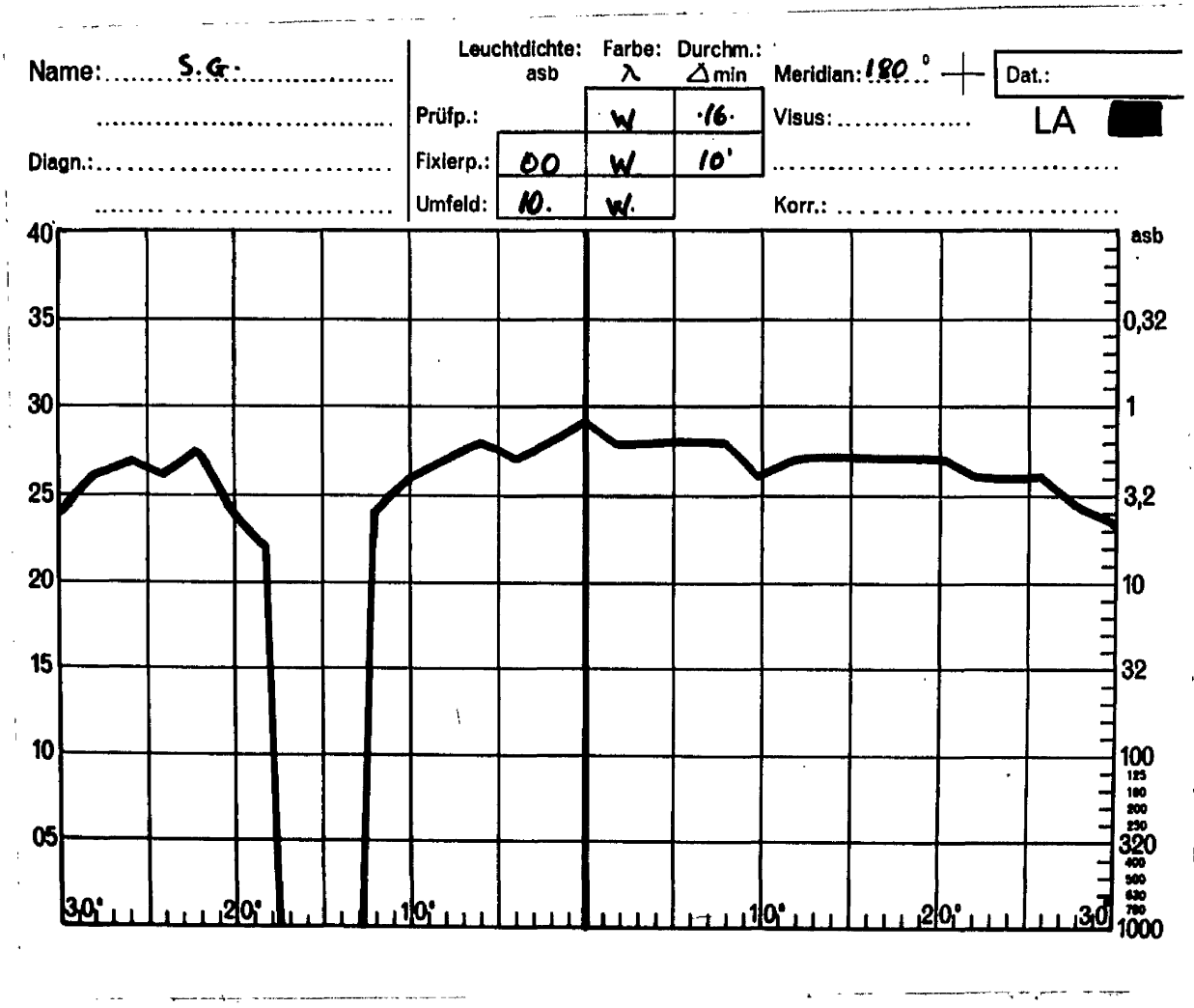


Figure 2. 11. Normal static perimetry chart across the horizontal meridian (180°) and cutting across both the fixation point and the blind spot.

found centro-caecal scotomas, that for red being larger than that for blue, and Heaton et al (1958) showed that the scotoma was bilateral, larger for colour than white, and accompanied by a temporal constriction for colour.

Evans (1939) found the following distribution of scotomata, confluent with the blind spot in 71; half way between blind spot and fixation point in 18; maximum central in 8. Bouniq and Coscas (1966) found almost an equal distribution between central and centro-caecal scotomas. According to Uhthoff (1886) the margin of the scotoma in the most severe cases may encroach into the periphery of the field and de Schweinitz (1922) found that the periphery of the field of vision was not always intact and defects could be found if the tests were carried out under diminished illumination. Hambresin and Schepens (1949) held the view that the scotoma of tobacco amblyopia and that of the hereditary optic atrophy were similar and they raised the question that the aetiology of both conditions was similar.

The patients in this analysis had examinations of their field of vision carried out by campimetry and static perimetry.

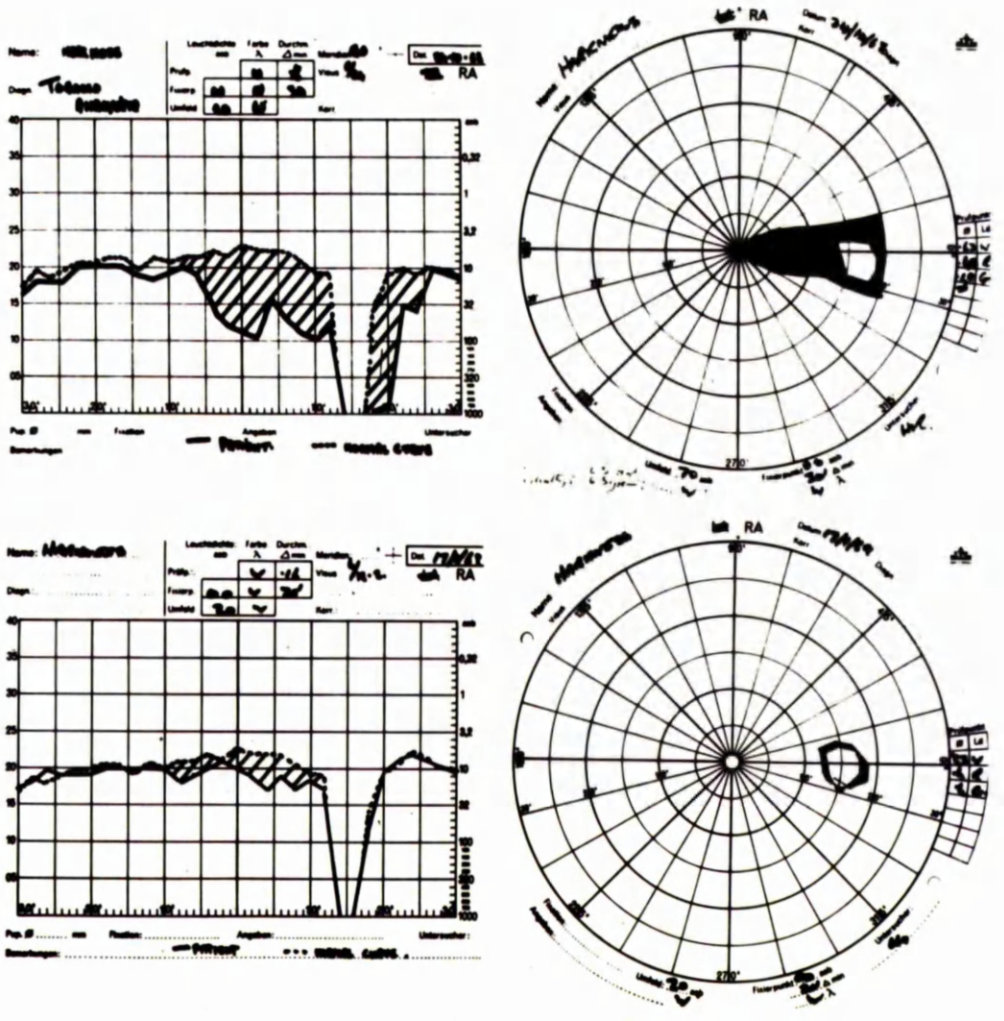


Figure 2, 12. Campimetry showing typical centro-caecal scotoma and static perimetry chart of untreated tobacco amblyopia (upper pair). Alteration in both campimetry and static perimetry after three months treatment with hydroxocobalamin (lower pair). Shaded area indicates extent of deviation from normal.

(a) Examination of the central area of the field of vision revealed typical centro-caecal scotomas before each eye in which the defect for colour was larger than that for white. In most patients the scotoma extended some way from the blind spot towards the fixation point, and in some, included the fixation point. Figure 2, 12 shows a typical example.

(b) The types of defect detected by static perimetry fell into 2 main categories and were similar to those described by Zingirian and Rivara (1965). The first type of defect is a relatively uniform slope and was present on examination of 8 eyes, the second is similar but the slope is interupted by a tooth or spike in the juxta caecal area and was present in 52 eyes out of a total of 60 eyes examined by this method. In all the cases examined the nasal margin of the defect extended beyond the fixation point for a variable distance into the nasal field, even in these cases who retained good visual acuity.

The response to therapy can be demonstrated by static perimetry and Wilson (1969) followed patients suffering from tobacco amblyopia, treated with hydroxocobalamin by recording the change in field area. A typical example of

the field defect recorded by kinetic and static perimetry before and after several months therapy with hydroxocobalamin is shown in Figure 2, 12.

Patient Age in Tobacco Amblyopia.

All the 65 patients were smokers; 57 pipe only, 3 cigarettes only and 5, both. There were 64 male patients and 1 female. The mean age of this group was 67± 9.1 years, with a range of 46 - 84 years; 47 of the patients (about ⅔) were of retiral age or older, but the greatest incidence lay in the 60-80 years age group. (As shown in table 2, 4). This is higher than the previously recorded incidences; maximum incidence in the 50's recorded by Usher (1927a) and Traquair (1930).

However, when the figures are related to the percentage of the male population in these age groups the incidence is found to be maximal in the 70-80 years age group.

.In this analysis the figures for the 1961 census for Glasgow were used. The proportion of males in each age group was compared, as a ratio Tobacco amblyopia/census. The

results are contained in table 2, 4 and figure 2, 13 and refer only to the male patients.

Table 2, 4.

Age Group	40-49	50-59	60-69	70-79	80+	Total
Numbers.	2	12	22	24	4	64
Percentage	3.12	18.72	34.32	37.44	6.24	
1961 Census	12.5%	12.45%	7.56%	3.44%	0.94%	
Ratio E.A. Census	0.25	1.5	4.54	10.88	6.64	

The proportion of smoking to non-smoking males has been examined by Todd (1966) who found an over all incidence for all ages of 72% smokers in 1961 and 68% in 1965, but indicated a falling incidence by age. A study carried out by Brett and Benjamin (1968) on an industrial population revealed an incidence of 68.2% smokers of whom 1.5% were pipe smokers. Doll and Hill (1964) found 13.2% of doctors were pipe smokers, and the Tobacco Research Council 5.4% of the general population (Todd 1966). Unfortunately, these published statistics do not show the incidence of smokers, by decades, above the age of 60 years.

Were the scatter of tobacco amblyopia patients

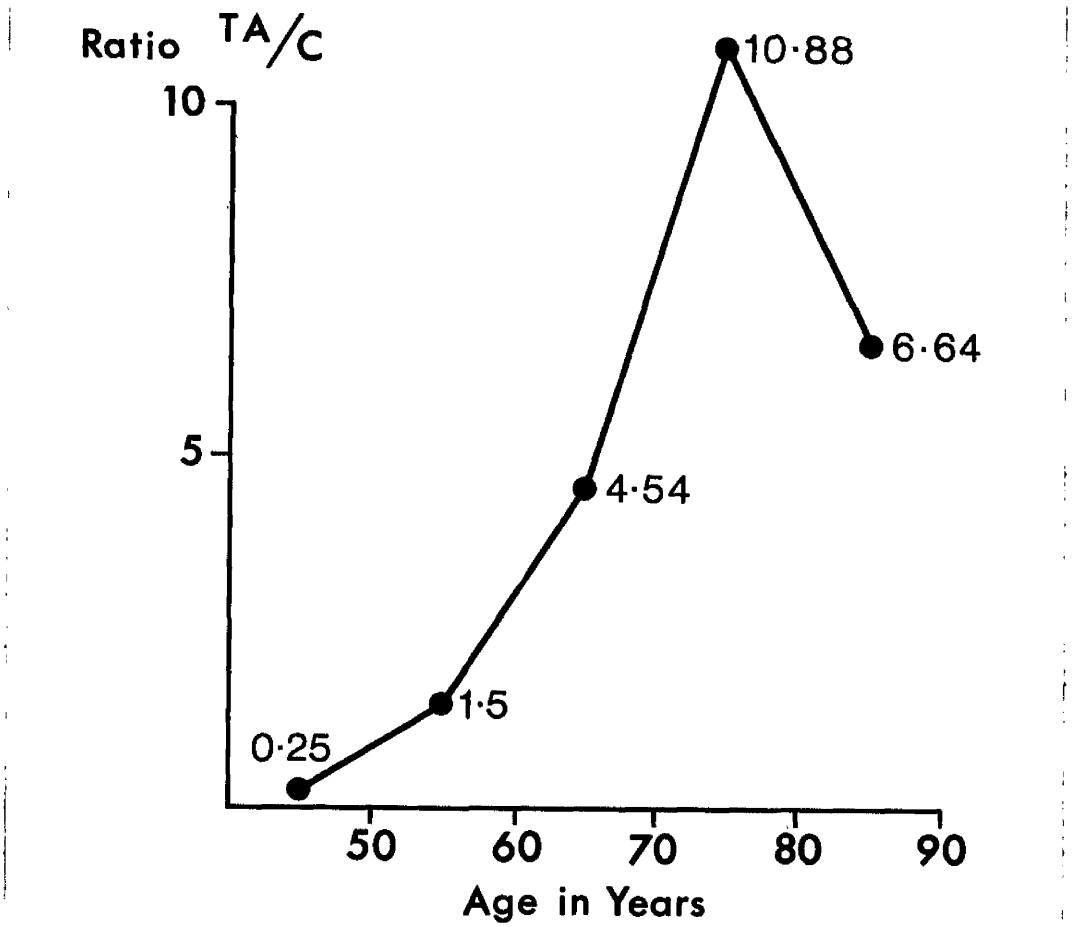


Figure 2, 13. Incidence of tobacco amblyopia with age, as indicated by the ratio of percentage of patients in experimental group to actual percentage of population in the same age groups.

evenly distributed with age the ratio Tobacco Amblyopia/ census would be the same in all age groups. The results not only confirm that tobacco amblyopia is a disease of the elderly, but also illustrate that the incidence of the disease increased with age up till 80 years, thereafter declining.

From the figures in this analysis it is evident that the incidence of the disease in the 70 year olds is 43 times as common than in 40 year olds.

Duration of Symptoms.

The duration of visual symptoms prior to seeking medical advice was known accurately in 55 patients and lay within the range 1 - 24 months with a mean at 6 months $\pm$ 4.5 months. Patients whose symptoms were present for an indefinite period have been excluded. Patients over the age of 65 years were as likely to report for treatment earlier or later than 6 months as were patients younger than 65.

	Visual symptoms $\leq$ 6 months	Visual symptoms for 6 months or more
Patients 65 years or younger	14	9
Patients older than 65 years	18	14

($x = 0.11$; $n = 55$; $p = 0.95$)

Thus age played no part in determining when a patient reported for advice.

Visual Acuity and duration of symptoms in untreated Tobacco Amblyopia.

A comparison between duration of symptoms and the percentage visual acuity yielded a correlation which was not significant ($r = 0.085$; $n = 55$; $p > 0.1$). Thus length of illness did not appear to materially affect the extent to which the visual acuity was lost.

Duration of Symptoms and Visual Outcome.

The effect of duration of symptoms on the visual outcome is dealt with on page 146 where it is demonstrated that the earlier the patient presents for advice the more likely that the outcome will be favourable.

CHAPTER III.

NUTRITIONAL FACTORS

It has been obvious to all observers that the majority of tobacco users do not develop tobacco amblyopia even when not in perfect health. Hutchinson (1873-87), Eales (1887) and Traquair (1930) postulated an individual inherited susceptibility to tobacco in patients showing tobacco amblyopia. General ill health (Eales 1887, Traquair 1930 and Krimsky 1934), and psychic shock (Traquair 1930, Hambresin and Schepens 1946) have been mentioned as precipitating causes. Nutritional disturbances have been blamed for an increased susceptibility to toxic amblyopia, in particular a vitamin deficiency, although Carroll (1935-56), Carroll and Franklin (1936) did not consider tobacco amblyopia purely the result of a deficiency state. The aetiologic factor of vitamin B_1 deficiency in the amblyopia of tobacco or alcohol habitues has been suggested by Duggen (1935), Gottlieb (1941) and Grosz (1938), but clinical trials have not convincingly shown that tobacco amblyopia is caused by vitamin B complex deficiency (Heaton et al 1958).

Sattler (1923), Bachtez (1920), Bachtez and Furtscher (1920), Traquair (1930), Hambresin and Schepens (1946) and Grosz (1949), have presented statistical evidence pointing to an increase in the incidence of tobacco amblyopia during wartime, particularly in countries suffering from food scarcities. There appears little doubt that the increase in tobacco amblyopia during the 1914-1918 and 1939-1945 World Wars was due to nutritional factors rather than by change in the degree or manner of tobacco use.

Carroll (1935) reported that the CSF in ten cases of tobacco amblyopia was essentially normal. De Schweinitz and Edsall (1903) studied the blood, urine, faeces and stomach contents and reported evidence of an upset in digestion and metabolism. Leishman (1951) found a high incidence of hypochlorhydria and achlorhydria in tobacco amblyopia. Heaton et al (1958) found a significantly low level of B12 in serum in tobacco amblyopia. They further pointed out that many workers have noted the importance of nutrition and general state of the patient with tobacco amblyopia.

Carroll (1935) felt that there were other factors

in the cause and progress of tobacco amblyopia other than tobacco and alcohol. He allowed patients to continue to smoke and consume alcohol while maintaining them on a high vitamin diet supplemented by yeast, wheat-germ, cod-liver oil, and liver extract injections. The success of his therapy is reflected in the rate of recovery which is as good as in any previous survey in which the patients had abstained from tobacco.

Influence of Alcohol.

Widely divergent views have been expressed concerning the influence of alcohol in the production of tobacco amblyopia. On the other hand, there are those who decline to Hutchinson's view (1876) that total abstainers from stimulants are more liable to suffer than others and that on the whole, alcohol counteracts tobacco. Thus, Berry (1887) held that alcohol more probably counteracts than abets the poisoning which gives rise to the amblyopia, and Gunn (1887) believed that total abstainers are probably peculiarly apt to get toxic amblyopia from a comparatively small amount of the poison.

de Schweinitz (1900) stated that alcohol was not

antagonistic to tobacco but, in fact, predisposed to tobacco amblyopia by producing a chronic dyspepsia. According to Uhthoff (1886-1901) alcohol alone in excess will cause the clinical picture of toxic amblyopia.

The following relationships between tobacco and alcohol in the production of the amblyopia have been suggested:-

(1) Both alcohol and tobacco are causative agents.

(2) Either agent alone is capable of causing the disease, although usually free indulgence in alcohol is associated with heavy smoking. (Ramsay 1895, Parsons 1901, Lyle 1903, de Schweinitz 1927, Lyle 1947 and Maxwell 1953).

(3) The influence of alcohol in the production of tobacco alcohol amblyopia is secondary or doubtful (Traquair 1930) since the amblyopia is observed in non-drinkers, but not in non-smokers. (Nettleship 1887, Hambresin and Schepens 1946). Tobacco-alcohol amblyopia improves when smoking is stopped even though the patient continues to drink (Berry 1882 and Evans 1939).

(4) Alcohol indirectly forms the toxic effect of tobacco on the eye (Berry 1887, Gunn 1887, Nettleship 1887 and Meaton et al 1959). Carroll (1935a) believed that

increased susceptibility of the patient was a far more important factor than the amount of tobacco or alcohol consumed. As a rule, neither tobacco or alcohol directly affect the eye, unless the substances have first attacked the general health (Krimsky 1934), a view which supports the belief that chronic retrobulbar neuritis is a true deficiency disease.

The alcohol intake of the present group was assessed in 55 of the patients; 16 patients abstained from alcohol, 18 occasionally took alcohol, and 21 had a daily intake of alcohol. The alcohol consumption of 8 patients was considered heavy, but in only 3 of this group did the visual improvement reach 6/9 (Snellen) with treatment. Of the 13 moderate drinkers 5 (38.4%) and of the 16 teetotal patients 10 (62.5%) achieved a visual improvement to 6/9 (Snellen), or better during the period of treatment. It would seem that the alcohol consumption influenced the outcome. However, it was found that those patients who drank heavily also smoked heavily:-

Heavy drinkers mean tobacco consumption 3.68 ozs per week.
Moderate drinkers mean tobacco consumption 2.6 ozs per week
Abstainers from alcohol mean tobacco consumption 2.5 ozs.

per week.

It was felt that alcohol played little if any part in the course of the illness.

Vitamin B12.

Since cyanocobalamin was isolated by Smith (1948) and the subsequent elucidation of its structure and that of various analogues, there has been considerable interest in their biological reactions. Vitamin B12 is a group of compounds, each of which is a complex porphyrin derivative containing a tervalent cobalt atom linked to a nucleotide. A nomenclature of the members of the Vitamin B12 group, based on the nature of the nucleotide composition has been proposed, in which the term Cobalamin is reserved for those compounds in which the nucleotide contains the base 5,6, dimethylbenzimidazole.

The six co-ordinate valencies of the cobalt atom are satisfied by the four nitrogen atoms of the tetrapyrrol, a nitrogen atom of the nucleotide; the sixth valency is taken up by cyanide in the case of cyanocobalamin (Figure 3,1)

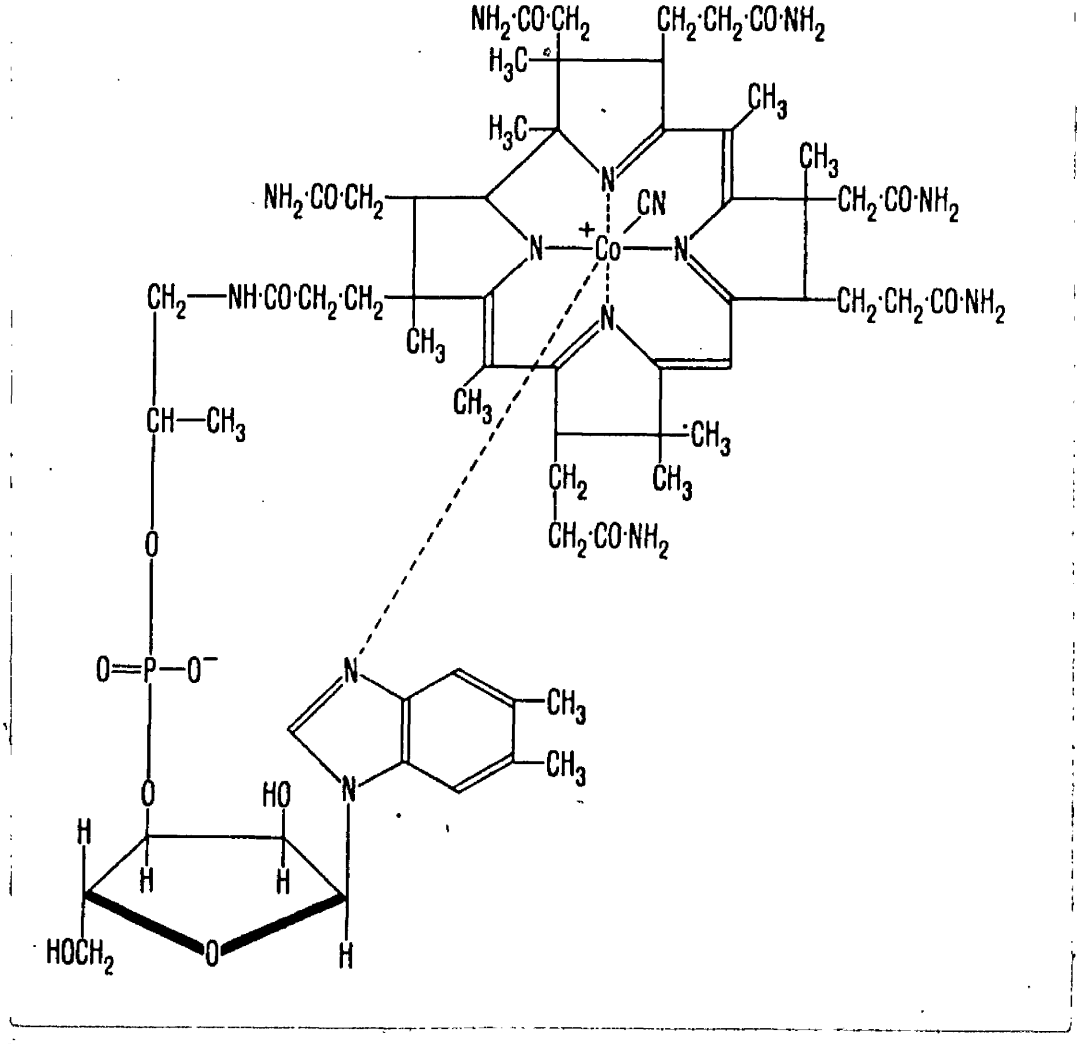


Figure 3. 1. Molecular configuration of Cyanocobalamin (5, 6, Dimethylbenzimidazolecobamide Cyanide).

by a hydroxyl ion in hydroxocobalamin, and by 5-deoxyadenosyl in co-enzyme B12. The manufacture of vitamin B12 is based on its direct microbiological synthesis by Streptomyces olivaceus or Bacillus megatherium. It is also obtained as a by-product of antibiotic manufacture (Johnson and Todd 1957).

Although cyanocobalamin was isolated from animal liver as the anti-pernicious anaemia factor, it has been subsequently shown that co-enzyme B12 is the major analogue in the liver (Toohey and Barker 1961), accounting for 80% of the vitamin B12 analogues, and is considered to be the active principle of vitamin B12 in the body.

1. Biochemical Considerations.

The carbon-to-cobalt bond by means of which the 5-deoxyadenosyl group is attached to the cobamide moiety in co-enzyme B12, accounts for the unique properties of this analogue. This bond may be split by the action of visible light, acid or cyanide (Weissbach et al 1960). Under anaerobic conditions, light causes homolytic cleavage of the carbon-to-cobalt bond yielding a reduced form of

cyanocobalamin. With the addition of air oxidation to hydroxocobalamin occurs (Brady and Barker 1961). The addition of acid splits not only the carbon-to-cobalt bond of coenzyme B12, but also the nucleotide bond, releasing free adenine and a sugar derivative (Brady and Barker 1961). Cyanide has somewhat similar effects forming not only cyanocobalamin, but also free adenine and a cyanohydrin.

Systems that can convert cyanocobalamin or hydroxocobalamin to deoxyadenosyl B12 have been described in bacteria (Weissbach et al 1962). The reaction requires A.T.P. which serves to transfer the 5-deoxyadenosyl moiety, reduced flavin and a sulphydryl compound; inorganic phosphate being released as a by-product.

A cobamine compound has been shown to be essential in some enzymatic reactions; the common active form being deoxyadenosyl B12.

(1) Isomerization of glutamate to - methylaspartate by the enzyme glutamate isomerase. (Barker et al 1958).

(2) In reactions involving hydrogen transfer under the influence of the enzyme glycol dehydrase. During this

reaction there is deactivation of the deoxyadenosyl B12. It has been suggested that this enzyme is decomposed to hydroxocobalamin, which in turn deactivates the glycol dehydrase, thus bringing about the observed slowing of the reaction. (Lee and Abeles 1963).

(3) Methane formation from Co2 = Carbon Dioxide, formate, methanol, serine etc. by macrobic bacteria, utilises methylcobalamin as a cofactor and intermediate metabolite.

(4) Biosynthesis of acetate from Co2 = Carbon Dioxide.

(5) Isomerization of methylmalonyl CoA to succinyl CoA by Methylmalonyl CoA isomerase is an important reaction in the metabolism of propionate. This reaction involves an intramolecular group transfer (Stadtman et al 1960).

(6) In Methionine synthesis there is a transfer of a methyl group from 5-methyltetrahydrofolate to homocysteine yielding methionine and tetrahydrofolate, thus explaining the relationship between vitamin B12, folic acid and carbon-one metabolism. The reaction takes place in three stages, the cobamide coenzyme is required only for stage C.

(a) Serine + tetrahydrofolate -- 5, 10, methylinetetrahydrofolate + glycine.

(b) 5, 10, methylinetetrahydrofolate -- 5, methyltetrahydrofolate

(c) 5, methyltetrahydrofolate - homocysteine -- tetrahydrofolate + methionine.

Reactions 1 - 4 have been observed in bacteria only (Weissbach et al 1964); the isomerisation of methyl malonyl CoA has been observed in mammals, and it is felt that methionine synthesis may also occur in mammals, though to date this has only been observed in photosynthetic bacteria.

2. Physiological Considerations.

Vitamin B12 can be synthesised by micro-organisms, bacteria, and actinomycetes. It does not occur naturally in yeasts, algae, or plants (Darken 1953); any vitamin B12 content present in them being obtained from sybiotic bacteria. Certain lower organisms require vitamin B12 as a growth factor (Ford and Luther 1955), while others can do without the vitamin (Burton and Lochhead 1951) or replace it with some other essential metabolite. More highly organised

animals depend on ingested vitamin B12 for their supplies of the vitamin and store it in their tissues, especially the liver, kidney, muscle, and in their milk and eggs.

Man depends on dietary sources for his supplies of vitamin B12. The vitamin is normally synthesised in large amounts by the intestinal flora, but apart from coprophagy this source is not utilised. The minimal daily requirements are not known with certainty. Although Ganong (1965) claims a minimal daily requirement of 2.8 µg., 1 ug. per 24 hours has been shown to be sufficient to prevent pernicious anaemia (Reisner and West 1949). Dietary vitamin B12 is absorbed in the terminal ileum after conjugation with the intrinsic factor mucoprotein secreted by the gastric mucosa. In man vitamin B12 is mainly stored in the liver, but large quantities are also found in the kidneys, the central nervous system and the myocardium. Vitamin B12 is present in serum either "free" or "bound". A small amount of vitamin B12 is normally present in serum as the "free" vitamin when it can be utilised by the tissues. The larger portion of the vitamin present in serum is "bound" to plasma protein (Pitney et al 1954); principally to alpha-globulin (73%), albumen (16%), beta-globulin (7%) and

gamma-globulin (4%) (Heinrich et al 1956). Normally the proteins are not fully saturated and can take up additional vitamin B12.

Hydroxocobalamin is firmly bound to the proteins generally, whereas cyanocobalamin is bound to specific globulins only (Miller and Sullivan 1958).

Boger et al (1955) studied the serum vitamin B12 concentration in a large group of normal healthy subjects. They used a biological method of assay utilising L. Leichmannii as the test organism. They discovered a negative correlation between serum vitamin B12 concentration and age. Analysis of plasma by chromatography has enabled the isolation of four vitamin B12 fractions in the blood, namely cyanocobalamin, hydroxocobalamin, deoxyadenosyl B12, and methylcobalamin (Lindstrand and Stahlberg 1963, Lindstrand 1964). Of these methylcobalamin was present in the largest proportion and Smith (1968) believes that this is the form in which vitamin B12 usually is transported within the body fluids.

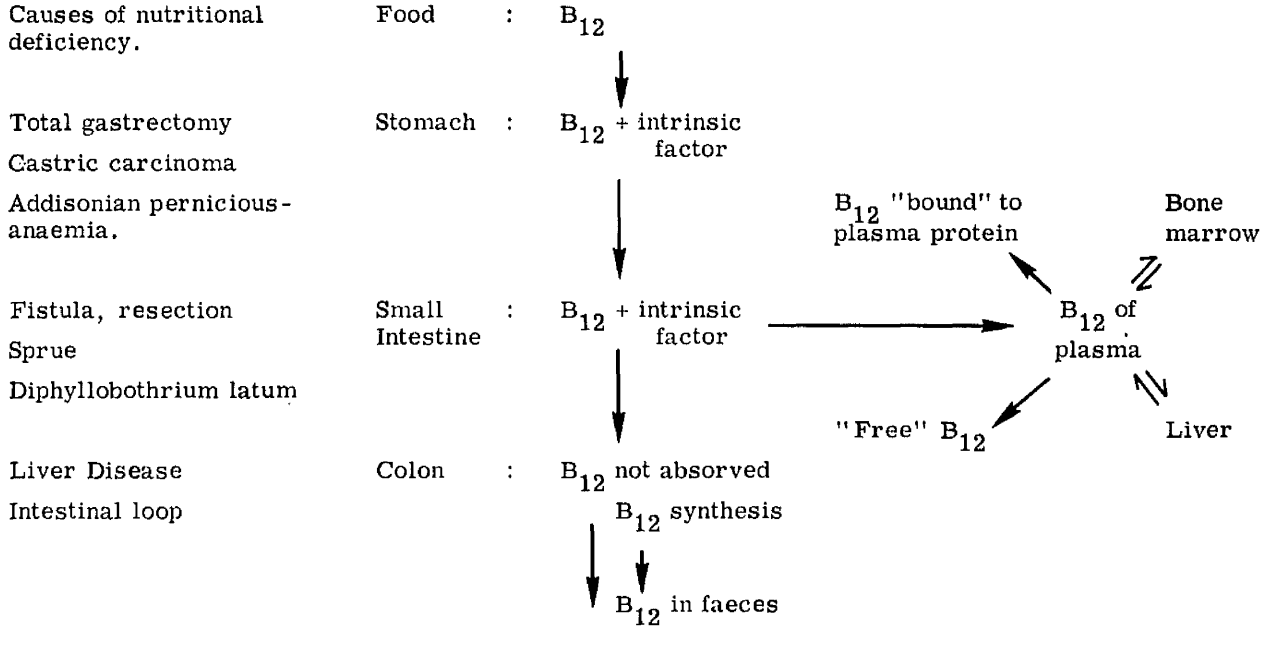
3. Patho - Physiological Considerations.

Lindstrand et al (1966) examined the plasma of a group of healthy smoking subjects. They did not find a significant rise in the level of the cyanocobalamin fraction, and felt that their results did not support the hypothesis proposed by Smith. Smith (1961) had found that the difference in assay levels, by L. Leichmanii, of serum vitamin B12 concentration with and without added cyanide, was less in smokers than non-smokers. He proposed that this was brought about by the presence of a higher concentration of cyanocobalamin in the plasma of smokers. This he claimed was part of the derangement in cyanide metabolism underlying tobacco amblyopia and related conditions. Linnell et al (1968) found that the urinary excretion of vitamin B12 and thiocyanate was raised in smokers and that the concentrations of these substances bore a positive relationship to each other, and further, the urinary thiocyanate concentration bore a negative relationship to serum vitamin B12 concentration. Thus a very high thiocyanate excretion may be associated with subnormal serum vitamin B12 concentration.

In hepatic cirrhosis Swendseid et al (1957) found that there was a greatly reduced concentration of

SUMMARY OF VITAMIN B12 DEFICIENCY STATES.

SUMMARY of B_{12} DEFICIENCY STATES in MAN



vitamin B12 in the liver, and Lear et al (1954) and Mackay et al (1957) that there was an increase in the "bound" vitamin B12 and often a fall of the "free" vitamin B12 in plasma.

It is well recognised that the serum vitamin B12 concentrations may be very low after partial or total gastrectomy and in association with the "blind" loop, (Badenoch et al 1955) and malabsorption syndromes, (O'Brien and England 1964), diverticulosis of the small intestine, treatment with anticonvulsants. (Spray and Witts 1958) and in the presence of helminthic infestations (Bjorkenheim 1966). Administration of intrinsic factor does not improve vitamin B12 absorption in patients with intestinal lesions. However, there may be an improvement in absorption after specific treatment, such as gluten free diet in adult coeliac disease (Mollin et al 1957), which may be of diagnostic value.

Becker et al (1954) have shown that diabetics have a tendency to vitamin B12 deficiency and this may explain the increased incidence of tobacco amblyopia in diabetics. Low serum vitamin B12 concentrations have been

reported in "vegans" (Smith 1962) whose dietary intake is low in foods containing vitamin B12. A dietary deficiency may be present in some elderly men, especially if they live alone (Read et al 1965) and this deficiency might be expected to predispose towards the development of tobacco amblyopia in such patients.

One of the effects of vitamin B12 deficiency is defective synthesis of nucleo-protein (Girdwood 1950), desoxyribonucleic acid in particular (Beck 1961), consequently many cells of the body are unable to form new cells at the normal rate, large cells being produced at a slower rate (Castle 1953). This is most apparent in tissues with a rapid turn-over of new cells, such as bone marrow. In pernicious anaemia the marrow cells show abnormalities in nucleic acid content which revert to normal on therapy with vitamin B12 (Glazer et al 1954). The deficiency of ribonucleic acid is particularly injurious to the axon cylinders of the spinal neurones which show progressive degeneration (Castle 1953). Mental disturbances in pernicious anaemia are common and vary from minor defects of memory to major psychoses (Smith 1960, Shulman 1967) and show benefit from treatment with liver or vitamin B12

(Ungley 1949); the improvement being rapid and coincident with belief of the anaemia (Walton et al 1954). Smith (1964) reported the occurrence of metabolic encephalopathy in a patient suffering from pernicious anaemia who whilst on treatment with cyanocobalamin developed a lung abscess. The improvement of the encephalopathy after 500 ug of hydroxocobalamin was taken by Smith to indicate the toxic effect of cyanide as the cause of the encephalopathy. A somewhat similar case of tobacco amblyopia complicated by lung abscess and coma, was reported by Bronte-Stewart et al (1968- appendix B4), which responded to cyanocobalamin thus indicating a deficiency of vitamin B12 rather than a toxic effect of cyanide as the dominant factor in the encephalopathy.

Methods of Examination and Results.

The patients in this analysis were investigated for avitaminosis B12 and, when possible, they were admitted to hospital for the tests to be performed. Estimations of the total serum concentration of vitamin B12, studies of the intestinal absorption of vitamin B12 by the Schilling test, and a general test for intestinal malabsorption by the

Xylose absorption test were carried out. In addition the serum concentration of folate was estimated, and the patients examined for evidence of coincident disease. An assessment of the dietary intake was carried out in some of the patients. In this section the results of these investigations are presented and their significance in relation to other factors is discussed. Examination of the peripheral blood and bone marrow were also carried out and their discussion will be found in the section on the Optic Neuropathy of Pernicious Anaemia.

A. Serum Concentration of Vitamin B12.

The serum concentration is believed to reflect the state of the body stores of vitamin B12. This concentration may be assayed by biological (Girdwood 1960), or radio-isotopic means (Matthews et al 1967). At present the biological assay is universally used and was the method used in the present group. The organisms that have been utilised for this type of estimation are the flagellate Euglena gracilis, Lactobacillus leichmanii a mutant of Escherichia coli and the protozoan Ochromonas malhamensis. Euglena gracilis possesses the greatest sensitivity for

vitamin B12, Ochromonas malhamensis the greatest specificity.

The estimations of the serum vitamin B12 concentration were carried out by the Euglena gracilis method of assay after the method of Ross (1952). This organism was first used by Hutner et al (1949) and applied to body fluids by Mollin and Ross (1952). The present assays were carried out by Doctor J.F. Adams, Southern General Hospital, Glasgow.

Serum Vitamin B12 concentrations in Tobacco Amblyopia.

This was determined in 62 of the 65 patients. The serum B12 concentrations lay within the range 15 pg - 572 pg/ml. with a mean at 196 ± 150.27 pg/ml. The serum B12 concentration was below 100 pg/ml. In 9 patients and below 150 pg/ml. in 23 patients. Taking values of 150 pg/ml. and below as abnormal, 26 of the amblyopes (40%) had reduced concentrations of vitamin B12 in the serum.

A comparison was made between the mean serum B12 concentration of a control group of 71 non-amblyopic subjects

and the amblyopes. The subjects of the control group were drawn from the hospital population attending with conditions other than amblyopia and who were over the age of 45 years. In age composition the groups matched ($t = 0.07$, $n = 134$, $p = > 0.1$) but the control group contained smokers and non-smokers.

	Age	B12	Numbers
Amblyopic Group	67 ± 9.1 years	169 ± 150.27 pg/ml.	65
Non-amblyopic group	67.3 ± 11.8 years	237 ± 119 pg/ml.	71

The mean serum vitamin B12 concentration in the amblyopic group was significantly lower than in the non-amblyopic group ($t = 2.90$, $n = 134$, $p < 0.01$) thus confirming the findings of Heaton et al (1958).

In an attempt to determine whether the low concentration of serum vitamin B12 found in 40% of the tobacco amblyopes was the result of tobacco smoking or an independently determined factor, the tobacco consumption in these patients was plotted against the serum vitamin B12 concentration (Fig. 3, 2). A significant but not very

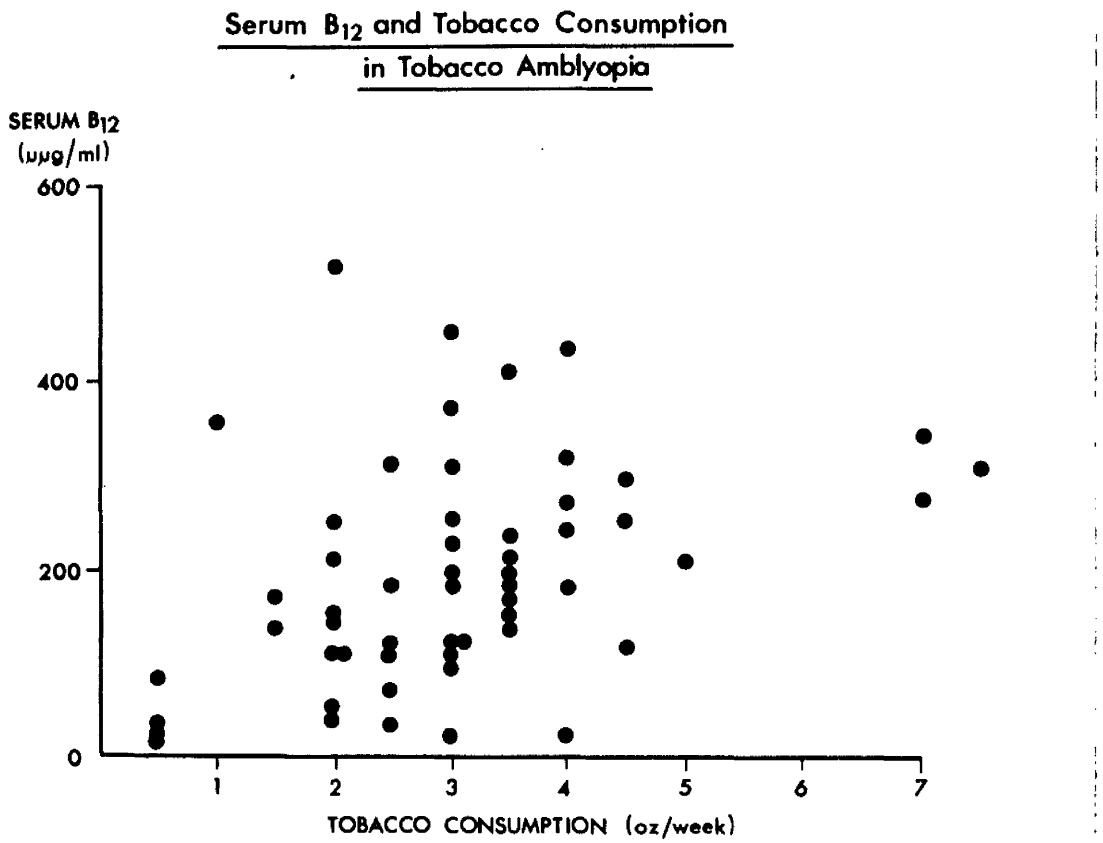


Figure 3, 2. The serum vitamin B12 concentration compared with tobacco consumption in untreated tobacco amblyopia. Correlation is just significant ($r = +0.38$, $p < 0.01$).

close positive correlation was found between these factors. ($r = +0.38$; $n = 53$; $p < 0.01$). Thus, tobacco amblyopia occurred with a low consumption of tobacco in those patients with a low serum vitamin B12 concentration, a much higher consumption of tobacco being the rule when the patient's serum vitamin B12 concentration was in the normal range.

To test whether the relationship between tobacco consumption and serum vitamin B12 concentration was common to all smokers or only amblyopes, the following groups were examined. Serum vitamin B12 assays and estimates of tobacco consumption were made in 28 male non-amblyopic pipe smokers; and 43 non-smoking subjects. Both groups were drawn from a hospital population undergoing treatment for eye conditions other than tobacco amblyopia (see table).

	Number in group	Age	Tobacco Consumption	Serum Vitamin B 12 Concentration
TOBACCO AMBLYOPIA	65	67 ± 9.1	3.04 + 1.44 ozs/week	196 ± 150.27 pg/ml.
NON-AMBLYOPIC PIPE SMOKERS	28	64.9 years	2.74 ± 1.32 ozs/week	269.5 ± 101 pg/ml.
NON-SMOKERS	43	68.8 years	-	228 ± 116 pg/ml.

(a)Tobacco consumption and serum vitamin B12 concentration for 20 male non-amblyopic pipe smokers of similar age composition to the amblyopic patients was plotted. The result is shown in Fig. 3. No significant statistical correlation was found between these two factors ($r = -0.001$). The mean tobacco consumption of this group was not significantly different from that of the amblyopic group of patients.

2.37 ± 1.3 ozs/wk in non-amblyopic group;
3.04 ± 1.44 ozs/wk in the amblyopic group
($t = 0.30$; $n = 91$; p 0.1)

(b) The mean serum vitamin B12 concentration in a group of non-smoking subjects was compared with that of the pipe-smoking non-amblyopic subjects. In the former group the assay was $228pg \pm 116$ pg/ml. and in the latter 269.5 ± 101 pg/ml. There is no significant statistical difference between the two groups. ($t = 1.55$; $n = 69$, $p > 0.1$). The mean serum vitamin B12 concentration in the non-amblyopic smokers is significantly greater than that of the amblyopic group ($t = 2.79$, $n = 91$, $p < 0.01$).

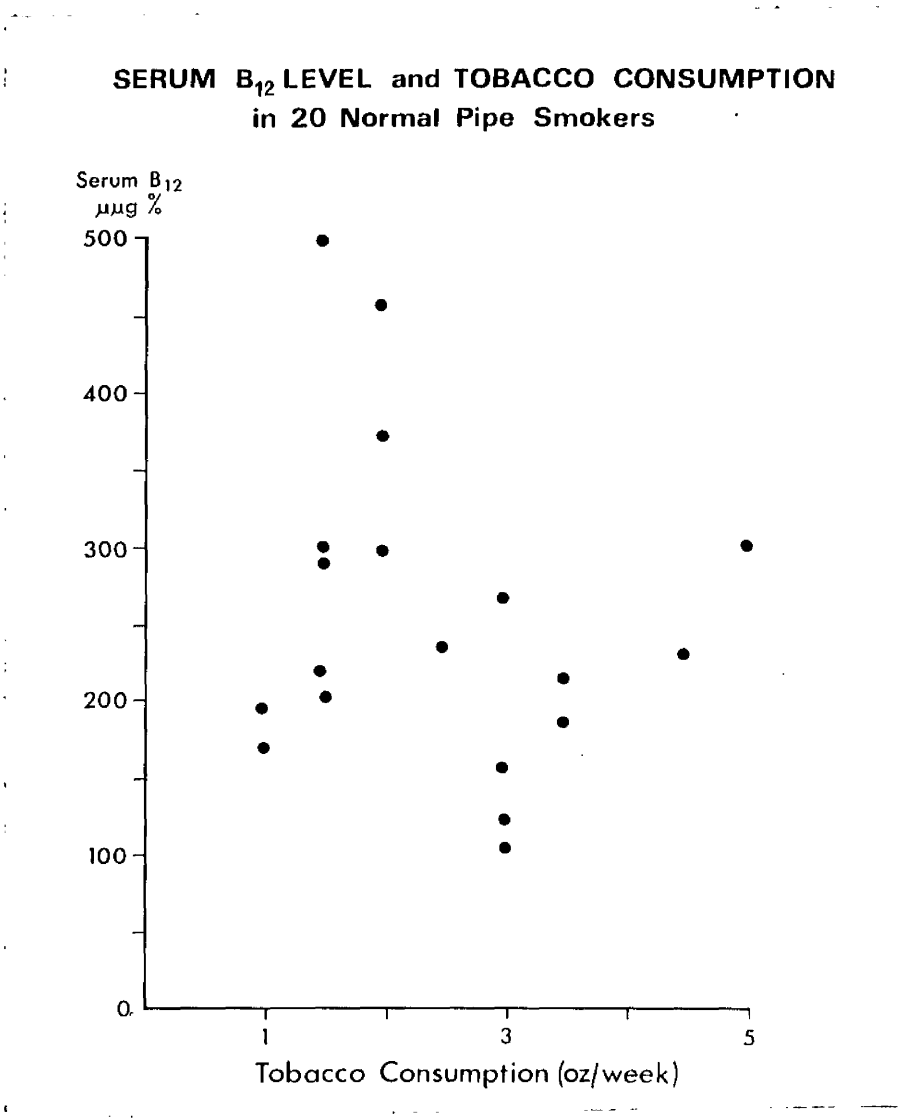


Figure 3. 3. The serum vitamin B12 concentration compared with tobacco consumption in non-amblyopic smokers (r = -0.001).

B. Intestinal Absorption Studies.

1. Vitamin B12 Absorption Test.

Malabsorption of vitamin B12 may be due to gastric lesions which impair the secretion of intrinsic factor, or to lesions of the small intestine which interfere with the absorption of the vitamin B12/intrinsic factor complex. Tests of vitamin B12 absorption using radioactive B12 have their most useful application in the diagnosis of pernicious anaemia, but are also useful for demonstrating intestinal malabsorption of vitamin B12, even when there is no other evidence of intestinal malabsorption.

The measurement of vitamin B12 absorption is essentially a two-stage procedure. The test is first carried out by giving the patient a small oral dose of radioactive vitamin B12. If the patient fails to absorb this orally the test is repeated with the addition of intrinsic factor. The absorption is assessed by one of the following methods:-

(a) Urinary excretion method (Schilling 1953).

(b) Faecal excretion method (Heinle et al 1952).

(c) Hepatic uptake method. (Glass et al 1954).

(d) Whole body radioactivity method (Reizenstein et al 1961).

(e) Plasma radioactivity (Booth and Mollin 1956).

In clinical practice the urinary excretion method is the most widely used; however the whole body radioactivity method or plasma radioactivity method is probably the more accurate.

Schilling tests were performed on 46 of the 65 tobacco amblyopia patients as follows:-

1. Patient fasted overnight.

2. Bladder emptied and urine discarded

3. Drank dose of radioactive vitamin B12 labelled with Cobalt 58.

4. After 2 hours 1 mg. hydroxocobalamin was given intramuscularly.

5. All urine was collected for 24 hours and the radioactivity present measured.

Values of less than 12% recovery were found in 21 tests (45.7%) and of less than 5% in 6 tests (13%). The Schilling test results were plotted against tobacco consumption. A similar trend to that found on comparing serum vitamin B12 concentration and tobacco consumption was revealed though the relationship was not so statistically significant ($r = +0.33$, $n = 40$, $p < 0.05$). The Schilling test results showed that in tobacco amblyopia, poor absorption of vitamin B12 tended to be associated with low levels of tobacco consumption and with test results within the normal range, tobacco consumption was high (Fig. 3, 4).

2. Xylose Absorption Test of Helmer and Fouts (1937).

This test was used as a general indicator of intestinal malabsorption. Xylose is a metabolically inert sugar and its rate of appearance in the urine is an indicator of its rate of absorption.

This test was carried out on 33 of the tobacco amblyopia patients as follows:-

1. Patient fasted overnight.
2. Bladder emptied and urine discarded.

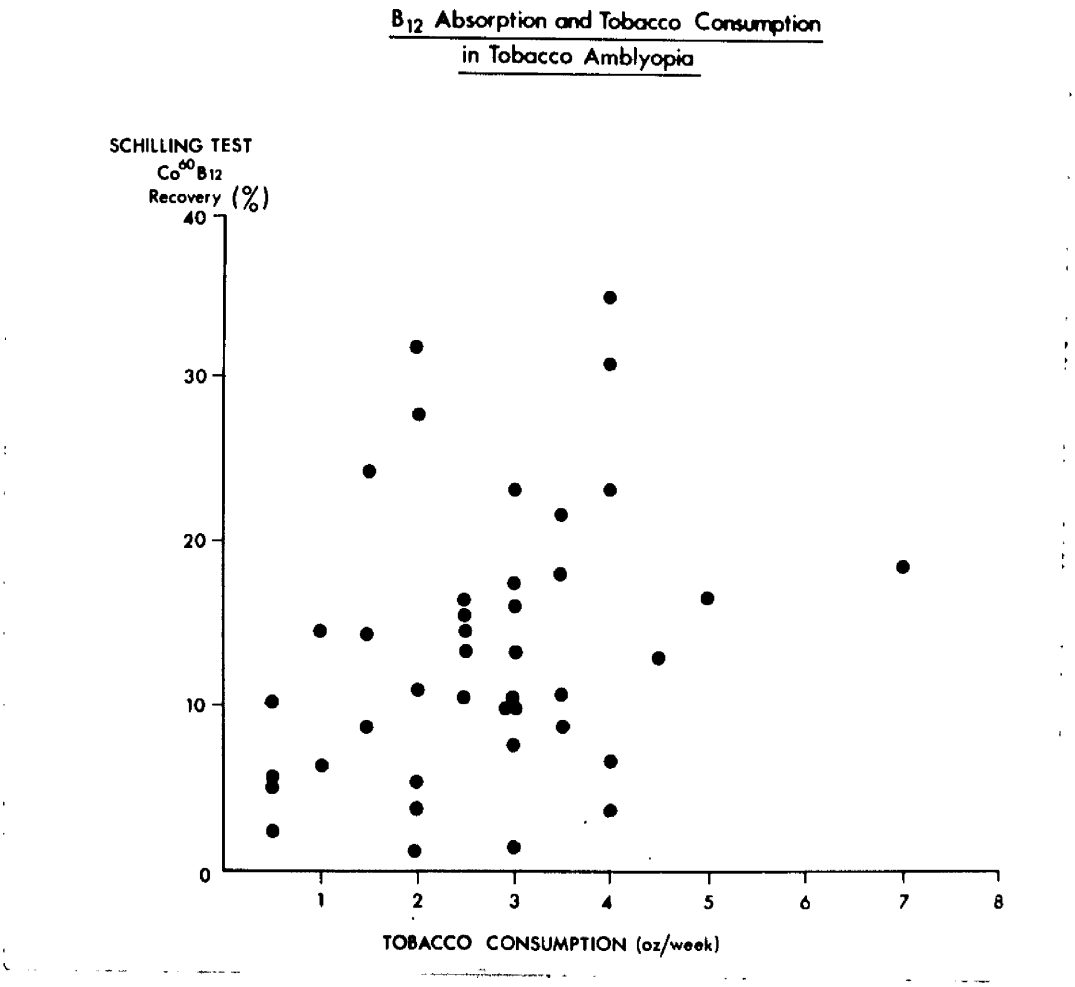


Figure 3. 4. Comparison between tobacco consumption and Schilling test result in untreated tobacco amblyopia ($r = +0.33$, $p < 0.05$).

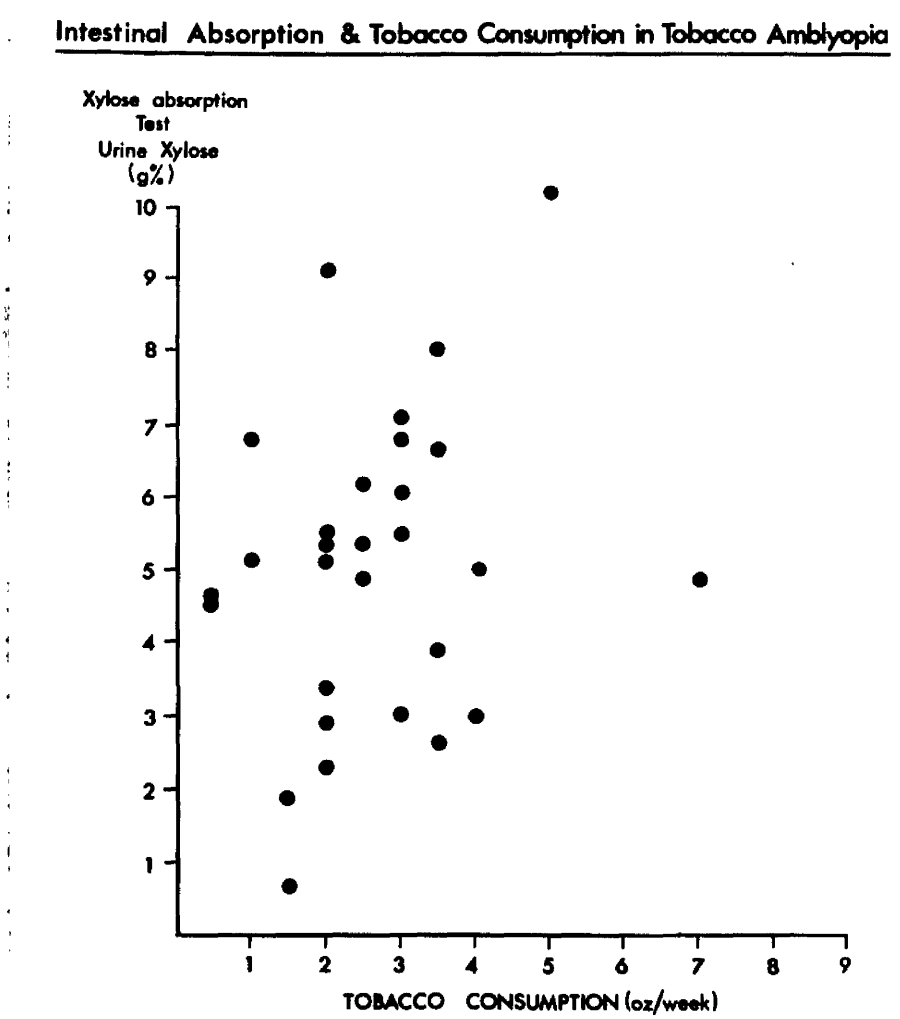


Figure 3, 5. Comparison between tobacco consumption and Sylose absorption test result, in untreated tobacco amblyopia. ($r = +0.234$, $p > 0.1$).

3. 5 gms. Xylose taken orally in 250 mls. of water. This was then followed by a second 250 mls. of water.

4. Patient was kept at rest and had nothing to eat or drink till end of test.

5. Blood sample was taken at 2 hours after oral Xylose.

6. Bladder emptied at 5 hours after oral xylose.

When the Xylose absorption test result was plotted against tobacco consumption a similar trend to that found on comparing tobacco consumption with serum vitamin B12 concentration, or the Schilling test was obtained. The statistical correlation however, fails to be significant. ($r = +0.234$, $n = 29$, $p > 0.1$). (Figure 3, 5).

The Xylose absorption test probably gives a measure of intestinal absorption, particularly in these patients whose faulty vitamin B12 absorption is not due to a specific defect such as Addisonian Pernicious Anaemia.

C. Serum Folate Concentration.

In contrast to the well recognised neurological abnormalities produced by a low concentration of vitamin B12 in the serum the effects of a lack of folic acid on the nervous system are relatively unknown. Reynolds (1968) suggested that folate deficiency was the cause of some of the mental changes seen in old age and that apathy led to a deficient intake of dietary folate. Girdwood et al (1967) were unable to confirm low folate concentrations in an elderly Scottish population.

Though dietary deficiency alone can produce megaloblastic anaemia (Jackson 1965), in Great Britain this type of anaemia is often associated with conditions which increase the requirement for folate, such as pregnancy (Gough et al 1963), psychiatric problems (Cairney 1967), ingestion of drugs (Malpas et al 1966), alcoholism and alcoholic cirrhosis, (McCurdy et al 1962, and Waters et al 1966), chronic renal disease (Sevitt and Hoffbrand 1969), Crohn's disease (Hoffbrand et al 1968), and rheumatoid arthritis (Gough et al 1964). The serum folate concentration is the earliest and most sensitive index of folate deficiency [Brit. Med. J., (1968), 2,378], and on this basis Read et

al (1965) and Rundle and Picton Williams (1966) found deficiency common in elderly patients in institutions.

Serum Folate Concentrations in Untreated Tobacco Amblyopia.

The serum folate concentrations were estimated by a biological method of assay after Waters and Mollin (1961) utilising Lactobacillus casei. One patient in this series was receiving therapeutic folate for a macrocytic anaemia due to alcoholic cirrhosis, and is excluded from the analysis. Serum folate concentrations were obtained in 51 patients:-

Of 9 patients with pernicious anaemia the mean folate was	8.28 ng/ml ± 2.89 ng/ml.
Of 42 patients without anaemia the mean folate was	6.17 ng/ml ± 3.06 ng/ml
Of all patients the mean folate was	6.43 ng/ml ± 3.28 ng/ml.

Pathologically low folate concentrations ($\leq$ 4 ng/ml.) were found in 10 patients (19%) of whom 1 had pernicious anaemia and 9 did not. A comparison between the mean folate concentrations in tobacco amblyopia complicated by pernicious anaemia and not complicated by pernicious anaemia showed little statistical difference. ($t = 1.757$, $n = 49$, $0.05 < p < 0.1$).

Although a comparison between age and serum folate concentration yielded a positive correlation which proved not to be significant ($r = 0.237$; $n = 49$; $p > 0.1$), a significant number of patients over the age of 65 years had serum folate concentrations in the subnormal range (below 6 ng/ml). ($x = 4.774$; $n = 49$; $0.02 < p < 0.05$).

In untreated tobacco amblyopia the serum folate concentration was compared with tobacco consumption in 51 patients. A positive correlation was obtained but this proved not to be significant. ($r = 0.074$; $n = 49$; $p > 0.1$). When the pernicious anaemia patients were excluded the correlation became more positive. ($r = 0.256$; $n = 40$; $p = 0.1$) Figure 3, 6.

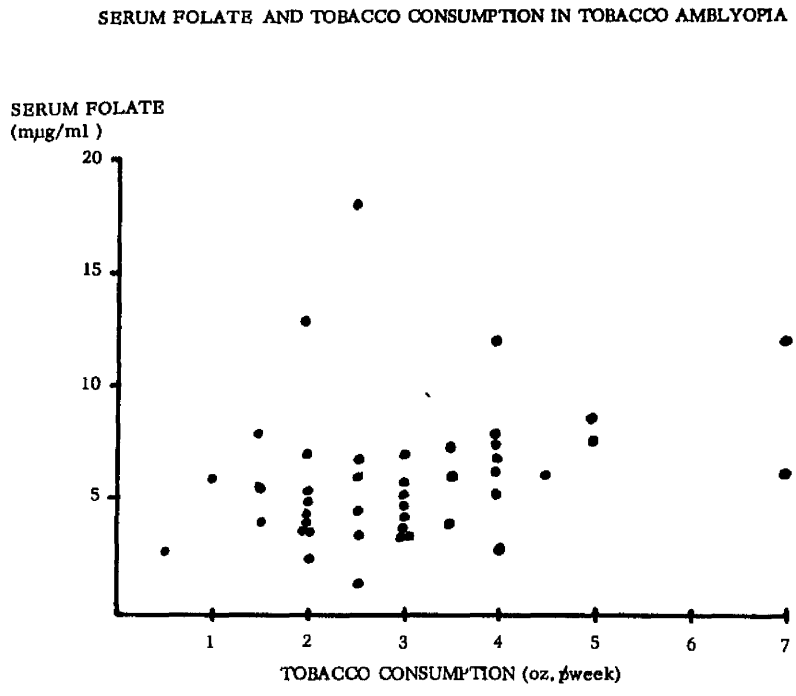


Figure 3. 6. Comparison between serum folate concentration and tobacco consumption in untreated tobacco amblyopia ($r = 0.256$). Patients with pernicious anaemia were excluded.

D. Dietary Vitamin B12.

Ganong (1965) claims that the minimum daily dietary requirement of vitamin B12 is 2.8 µg. Heinrich (1968) has shown that the turnover rate of vitamin B12 in the body metabolic pool is 0.05% (approximately 2.55 µg per day). This daily faecal and urinary excretion represents the optimal nutritional requirements. The minimum vitamin B12 requirement of 0.5 - 1.0 µg per day is just sufficient to satisfy the needs for normal erythrocytopoiesis and central nervous system function, but does not normalise vitamin B12 levels in the serum and tissues. If the daily metabolic turnover is not compensated for only the vitamin B12 pools in the storage organs and tissues can be used as a source for the metabolic vitamin B12 requirement. Thus, in face of reduced intestinal absorption of vitamin B12, there is progressive depletion of coenzyme B12 stores and a reduction in the serum concentration of vitamin B12.

With the aid of the hospital dietician the diets of 13 patients were analysed for vitamin B12 content. When the vitamin B12 content of uncooked food was estimated, the mean daily intake was 4.04 ug per day; 7 patients having

an intake of 2.5 ug per day or less. Assuming a 50% loss through cooking, the number of patients whose diet contained 2.5 ug per day vitamin B12 or less, rose to 9 (69%). Of these patients 5, (38.5%) were below the minimal daily requirement of 1.0 ug. outlined by Heinrich (1968). These figures confirm that the majority of tobacco amblyopes diet is low in foods containing vitamin B12.

B. Coincident Disease.

As predisposing to tobacco amblyopia the following diseases have been cited - digestive disorders (de Schweinitz and Edsall 1903), diabetes (Nettleship and Edmonds 1883, Carroll 1956, Doggart 1959), syphilis (Bussy 1926), and malnutrition (Berry 1884, Hales 1887, Lyle 1905, Hambresin and Schepens 1946, Grosz 1949, Doggart 1959). Leishman (1951) found that tobacco amblyopia and the optic neuropathy of pernicious anaemia could co-exist.

Examination of the patients investigated revealed the following systemic diseases:-

(a) Addisonian Pernicious Anaemia 11 (16.94%)

(b) Pre Pernicious Anaemia	11	(16.94%)
(c) Diabetes	6	(9.24%)
(d) Hepatic disease	2	(3.08%)
(e) Skeletal system disease		
Padget's disease of bone	1	(1.54%)
Rheumatoid arthritis	1	(1.54%)
Chronic osteomyelitis	1	(1.54%)
(f) Squamous carcinoma of skin	1	(1.54%)

No evidence of other complicating disease in 31 (47.74%).

CHAPTER IV.

THE TOXIC FACTOR IN TOBACCO

The Role of Tobacco.

All classic writers attributed to tobacco its full share of causation as a source of amblyopia (Wordsworth 1863). The opinions of later writers (Nettleship 1887, Lautenbach 1898, Gottlieb 1941, and Doggart 1959) that tobacco as a cause of toxic amblyopia (but not necessarily the only agent capable of producing this condition) were not synonymous with proof. The observation that recovery from tobacco amblyopia could lie in abstinence from tobacco would seem to implicate tobacco in some way (McKenzie 1854). Leishman (1951) was of the opinion that some noxious agent associated with tobacco, other than nicotine, would appear to be the ultimate source of tobacco amblyopia, and Hambresin and Schepens (1946) considered that tobacco amblyopia was only one symptom of chronic tobaccoism. Lautenbach (1898) considered tobacco amblyopia occurring in smokers to be induced not by the direct absorption of nicotine, but by the volatile products of combustion; but this could not explain tobacco amblyopia observed in tobacco chewers and

snuff takers. Traquair (1930) claimed that the exciting cause of tobacco amblyopia was tobacco, but the disease was determined by a depression in the patient's health, or a nutritional deficiency especially of vitamins. Thus, in persons with even mild vitamin B12 deficiency the retina or optic nerve would become unduly sensitive to the effects of tobacco (Heaton et al 1958). Mann (1908) after enumerating various substances found in tobacco smoke, stated that recent investigations pointed to nicotine as the sole effective poisonous constituent and referred to Habermann and Ehrenfield who had stated that in tobacco smoke nicotine was by far the most important component, the others including pyridine were of no importance. Usher (1927a) examined various tobaccos by estimating the nicotine content; of the brand of tobacco smoked by 92.9% of his amblyopia cases the nicotine content was 3 - 3.07%.

Bontly and Berry (1959) in their review state that early researches on cigar smoke reported the isolation of pyridine (C_5H_5N) and its analogues. Arsenic appears to have engaged the attention of many early workers but Lehmann and Gundermann (1912) were first to discuss cyanide. They found hydrogen cyanide and hydrogen thiocyanide in both

the gaseous and particulate phase of tobacco smoke from acid and alkaline tobaccos; cigar smoke containing more cyanide than cigarette smoke (Scholler 1935). Osborne et al (1956) and Johnstone and Plimmer (1959) examined the gaseous phase of cigarette smoke by infra-red methods, and found cyanide present in the condensable fraction to the amount $9.5 - 31.0 \times 10^{-2}$ mole%. The U.S. Surgeon General (1964) reported a tobacco smoke cyanide content of up to 1,600 p.p.m. These are high values indeed in the light of Glaister's (1953) statement that an atmosphere containing 3,000 p.p.m. of cyanide was lethal to homo sapiens. A recent report (Darby and Wilson 1967) indicated that there was little variation in the smoke cyanide content of various brands of pipe tobacco. These authors further conclude that the association between certain brands of tobacco and tobacco amblyopia was dependent not on difference in neurotoxicity but on popularity and cheapness.

Tobacco Consumption.

Usher (1927a) from his analysis of 1100 cases of tobacco amblyopia collected in Aberdeen, concluded that the majority of tobacco amblyopes smoke rather less tobacco than

non-amblyopic smokers. His figures were 2.65 ozs. per week for the amblyopes as against 2.72 ozs. per week for the non-amblyopic smokers when the total numbers were considered, but when only subjects over the age of 50 years were considered, the average consumption for the amblyopic group fell to 2.56 ozs. per week, and the non-amblyopic group to 2.60 ozs. per week.

The mean tobacco consumption of the present group of patients suffering from tobacco amblyopia is 3.04 ± 1.44 ozs. per week with a range of 0.5 ozs. to 7 ozs. per week. This group comprises both pipe and cigarette smokers, 57 pipe only, 3 cigarettes only and 5 both. The tobacco consumption in the amblyopic group is significantly higher then that of a comparable group of 37 non-amblyopic smoking subjects drawn from a hospital population, in which it is 1.85 ± 1.19 ozs. per week ($t = 14.39$; $n = 75$; $p < 0.001$).

The following scheme was used to convert cigarettes smoked per day into ounces of tobacco per week. The average weight of tobacco contained in 20 different brands of cigarettes, plain or tipped, was

found to be 0.7665 gm. (Sunday Times, 8th June, 1969).

5	cigarettes	per	day	=	1	oz.	tobacco	per week
10	"	"	"	=	2	oz.	"	" "
15	"	"	"	=	3	oz.	"	" "
20	"	"	"	=	4	oz.	"	" "
25	"	"	"	=	5	oz.	"	" "

Cyanide Detoxication and Excretion

Because of its highly reactive nature, and difficulty in its detection in body fluids, Wilson (1965a) concluded that an investigation of cyanide metabolism must concentrate on the products of that metabolism. Although cyanide is mainly converted to thiocyanate, the estimation of thiocyanate in body fluids is not an accurate index of cyanide exposure, because of alimentary absorption of dietary thiocyanate and variable concentration in body fluids. Amongst the factors which affect the concentration of thiocyanate the following are recognised:-

(1) Dietary intake of preformed thiocyanate contained in green vegetables and milk, is subjected to

seasonal variations (Stoa 1957).

(2) Deficient intake, as in anorexia nervosa and prolonged vomiting, is associated with low concentrations of thiocyanate in plasma (Stoa 1957).

(3) Defective absorption of oral thiocyanate has been observed in patients suffering from gastric carcinoma (Langman et al 1966).

(4) The Urinary excretion of thiocyanate tends to vary directly with urine volume and with chloride excretion (Stoa 1957).

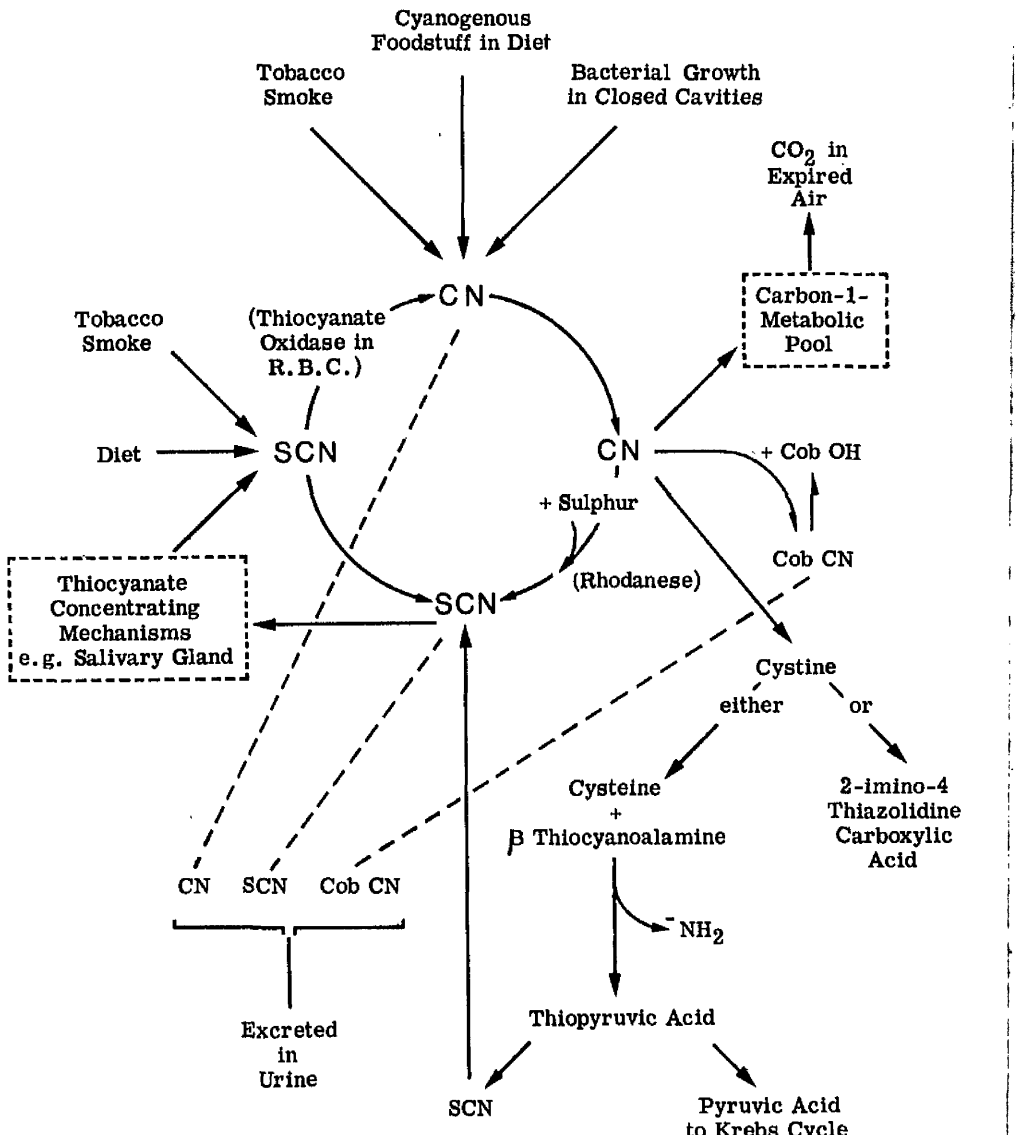
(5) Cyclical changes in thiocyanate concentration of plasma have been found to coincide with the menstrual cycle (Stoa 1957). These alterations are probably related to the state of hydration.

Naturally occurring cyanide and thiocyanate are found in foodstuffs such as cabbage and spinach (Stoa 1957), in dairy produce and milk (Wokes et al 1952), in alcoholic beverages such as beer and stout (Stoa 1957), and in tobacco leaf and smoke (Lehmann and Gundermann 1912). Cyanide is produced endogenously from thiocyanate by enzymic action and is a by-product of bacterial growth in closed cavities.

That the major portion of exogenous or endogenous cyanide is excreted in the urine as thiocyanate was first described by Lang, S. (1894) and later by Smith and Malcolm (1930). The identification of the enzyme system was established by Lang, K. (1933). The enzyme was called Rhodanese and it has been found to be specific for free cyanide without action on organically bound cyano-groups. In this context it competes with hydroxocobalamin which takes up cyanide avidly to form the harmless cyanocobalamin (Smith 1968). Recent studies on the distribution of rhodanese in the tissues, show it to be widely distributed in the body, activity being greatest in the liver (Himwich and Saunders 1948 and Saunders and Himwich 1950).

As well as a mechanism for converting cyanide to thiocyanate, there exists a reverse pathway which mediated through Thiocyanate Oxidase (Goldstein and Rieders 1953). This enzyme is found in red blood corpuscles. The presence of thiocyanate oxidase, which ensures a persistent trace of cyanide in the blood, is confirmatory evidence that the latter in minute amounts is an essential metabolite (Smith

SUMMARY OF CYANIDE MECHANISMS



et al 1963), and is responsible for the positive relationship between plasma cyanide and thiocyanate concentrations found in non-smokers and healthy smokers by Wilson and Matthews (1966).

Patty (1921) observed that organisms grown on stagnant protein produced cyanide in quantity. This was especially true of the pyocyaneus and coliform organisms. Thus cyanide may be produced as a by-product in abscess formation, in urinary diverticula, or in the blind-loop intestinal syndrome (Ungley 1955, Smith et al 1963).

Boxer and Rickards (1952) concluded from their studies on the metabolism of the carbon of cyanide and thiocyanate that cyanide carbon enters the carbon-one metabolic pool. Their studies made use of radio-active carbon as a tracer. Cyanide injected into the experimental animal appeared in the urine as free cyanide, thiocyanate, and vitamin B12 bound cyanide (cyanocobalamin). Cyanide and thiocyanate were found to be in dynamic equilibrium as demonstrated by the activity of hydrogen cyanide in expired air following the administration of labelled cyanide and thiocyanate. Activity was also demonstrated in choline

and methionine, and in the intermediate formate; activity was also observed in liver proteins. These authors further state that the oxidation of cyanide to carbon dioxide occurs to a large extent.

Baumann and associates (1933) concluded that the thyroid gland had no part to play in the conversion of cyanide to thiocyanate but it had in the de-methylation of acetonitrils, thus accounting for the increased excretion of thiocyanate on its administration. The thyroid gland along with the salivary glands form part of a concentrating mechanism for thiocyanate. Clemedson et al (1960) demonstrated active secretion of thiocyanate into the gastric juice, and deposits of thiocyanate in the walls of the major blood vessels by means of auto-radiographic studies.

Cyanide Demyelination.

Wilson and Matthews (1966) thought it possible that relatively low (though physiological) vitamin B12 concentrations may reduce the body's ability to detoxicate cyanide and result in relatively high blood concentration

of this radicle. Such concentrations may contribute to the pathogenesis of the neurological complications of vitamin B12 deficiency as well as the reported abnormalities of pyruvate tolerance (Hornabrook and Marks 1960). Over the past 60 years much research effort has been put into solving the mechanism of cyanide demyelination without concrete success. The earlier workers used relatively large doses of cyanide and the neurological lesions were diverse and inconstant, and could not be separated from the effect of anoxia. (Collins and Martland 1908, Hurst 1942, Hicks 1950, Lumsden 1950, Rose et al 1954, and Levine and Stypulkowski 1959). That cyanide in small doses was capable of producing foci of demyelination in a constant and reproduceable manner was shown by Smith et al (1963) and Smith and Duckett (1965). It has further been demonstrated that the liver stores of vitamin B12 in the experimental animal were depleted on chronic cyanide administration (Braekkan et al 1957), and that over a long period the ability to excrete the bulk of the administered cyanide as thiocyanate was lost (Smith and Duckett 1965). Mushett et al (1952) have shown that hydroxocobalamin protects the laboratory animal against lethal doses of

cyanide, a property confirmed by the studies of Smith et al (1963) and found not to be shared by cyanocobalamin (Smith and Duckett 1965). The latter researchers felt that hydroxocobalamin in some way preserved the integrity of myelin, as there was no evidence of demyelination in a group of animals subjected to chronic cyanide administration covered by hydroxocobalamin such as there was in a comparable group of rats in whom the cyanide was covered with cyanocobalamin.

Cystine, a highly insoluble amino acid, is capable of passing freely through the blood-brain barrier and becomes incorporated into the neural proteins (Ford et al 1961). Cyanide combines with cystine to yield 2-imino-4-thiazolidinecarboxylic acid both invivo and in vitro (Wood and Cooley 1956). These authors further found that the expected reaction on combining cyanide with cystine to yield cysteine (Voegtlin et al 1926), and B-thiocyanoalanine did not occur under normal physiological conditions in the experimental animal. The thiocyano-alanine would yield thiocyanate after deamination to thiocyanopyruvic acid and subsequent decomposition. This

conversion may indicate an alternative pathway of cyanide detoxication. The importance of 2-imino-4-thiazolidinecarboxylic acid in relation to demyelination is as yet unknown.

Methods Used in the analysis of cyanide and thiocyanate in Serum, Plasma and Urine.

In a critique of the methods for thiocyanate determination Stoa (1957) observed that the Pyridine-Benzidine reaction was the method most suitable for the determination of thiocyanate in blood serum. As a routine method of serum analysis the pyridine-benzidine method cannot be rivalled, as the results obtained at low concentrations of thiocyanate were as reliable as those at high concentrations. This property was not shared by the Ferric nitrate method which is not accurate at low concentrations.

In the earlier part of this analysis the Ferric nitrate method of Bowler (1944) was used as this was the only method available. Later when in receipt of a Medical

Research Council grant, determinations were carried out after pyridine-benzidine method of Aldridge (1945). In this subsequent portion of the analysis, the amendments of Wilson (1963-65) to the Aldridge method, and the aeration technique of Boxer and Rickards (1952), were followed.

(a) Colorimetric analysis of Ferric Thiocyanate (Bowler 1944).

To 1ml. of serum in a test tube, 6.5 ml. of water and 2.5 ml. 20% Trichloracetic Acid are added. Mix and stand for 10-15 minutes. Filter. To 5 ml. filtrate add 5 ml. ferric nitrate acid reagent. Read in photo-electric colorimeter using transmission of wavelength 470 m u. From a standard curve the optical density readings are converted to milligrams thiocyanate.

Ferric Nitrate - Nitric acid reagent is prepared by dissolving Ferric Nitrate in 2 N nitric acid. Standard curve is prepared by measuring the optical density of known concentration of potassium thiocyanate solutions.

(b) Colorimetric analysis of Cyanogen Bromide (Aldridge 1945).

1. 1 ml. sample (deproteinised plasma, or urine).
   +0.5 ml. $\frac{N}{1}$ Hydrochloric Acid.
2. 0.1 ml. (2 drops) solution of Bromine water. This converts cyanide and thiocyanate to cyanogen bromide.
3. 0.1 ml. 2% arsenious acid - removes excess Bromine. Shake well.
4. Drew air off surface of liquid with water pump to remove any Bromine vapour present.
5. Add 1.8 ml. of fresh Benzidine-pyridine mixture.
6. Leave 15-20 minutes.
7. Read in spectro-photometer at a transmission wavelength of 532 m μ. The optical density reading is converted to micro moles (um) per 100 mls. from a standard curve prepared from known concentrations of potassium thiocyanate.

2% arsenious acid is prepared by dissolving arsenious oxide in sodium Hydroxide solution. The solution is then rendered acidic by back titrating with acid using phenophthalein as indicator till ph 7 is reached.

The Benzidine-pyridine solution is freshly prepared by mixing a solution of pyridine with a benzidine

solution in proportions 5 : 1.

(a) Pyridine solution in Hydrochloric acid is prepared in the following proportions - Pyridine : Conc. Hydrochloric acid : water - 6 : 1 : 4.

(b) Benzidine solution is prepared as a 2% w/v soln. in Hydrochloric acid.

Analysis of urine for thiocyanate is carried out as follows:-

1. A 20 ml. aliquot of urine is diluted in 200 ml. with distilled water. 1 ml. samples are taken for thiocyanate estimations after the method of Aldridge.
2. No further preparation of the urine is required unless protein is present. The protein must be removed by precipitation with 10% Trichloroacetic acid before dilution is carried out.

(c) <u>Deproteinisation of plasma.</u>

1. 10 ml. blood placed in a heparinized tube.
2. Centrifuge at 3,000 r.p.m. for 30 minutes.
3. 5 ml. of supernatant plasma are then mixed thoroughly with 10 mls. 10% Trichloracetic acid, allowed to stand

for 10 minutes and then centrifuged for 30 minutes.

4. Supernatant is then decanted into a 50 ml. volumetric flask, or an aeration tube.

5. 5 ml. 10% Trichloracetic acid are then mixed with the residual protein coagulation and centrifuged for a further 10-15 minutes.

6. The supernatant is decanted as before.

7. Steps 5 and 6 are now repeated two further times.

8. The volume of supernatant fluid is now 30 mls. This is either diluted in the volumetric flask to 50 mls. by the addition of distilled water, 1 ml. samples being used for thiocyanate estimations after the method of Aldridge, or the 30 mls. are used for the estimation of plasma cyanide.

(d) Method of Concentrating Cyanide.

The method of Aldridge makes no distinction between thiocyanate and cyanide. The cyanide being volatile is removed by the aeration technique of Boxer and Rickards (1952), as follows:-

1. The sample before dilution, 30 mls. of deproteinised plasma, is placed in an aeration tube.

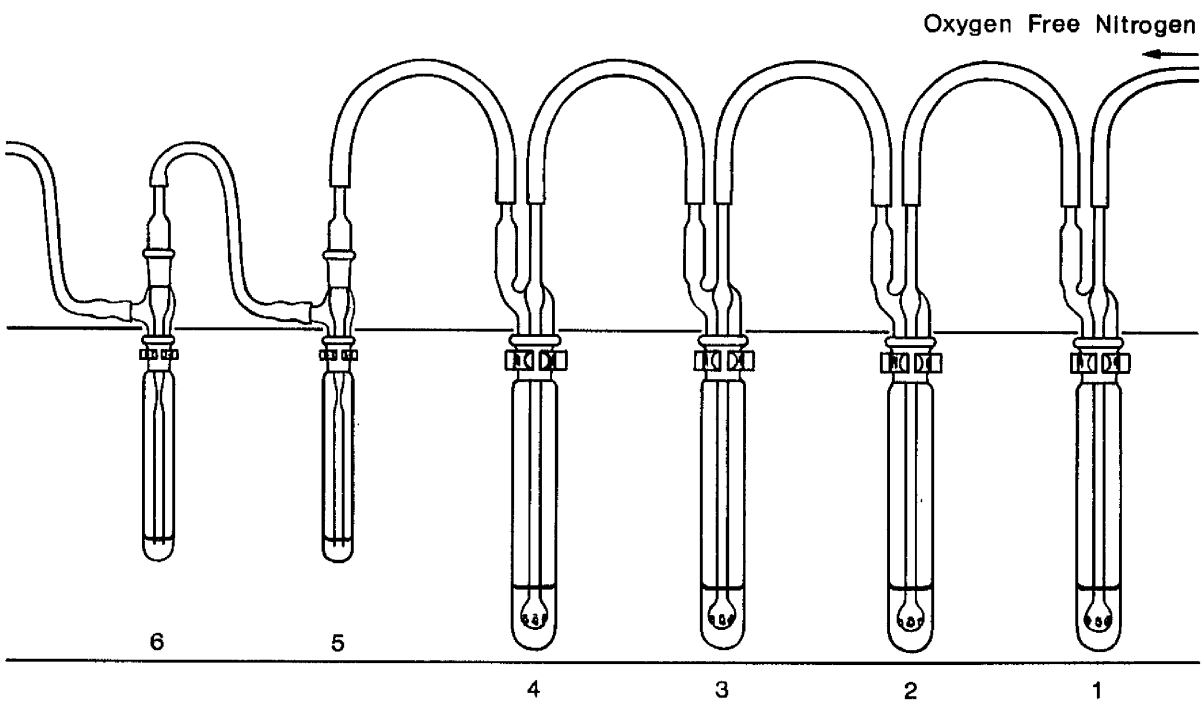


Figure 4,1. Illustrating the aeration train required to separate Cyanide from sample after Boxer and Rickards (1952).

2. This tube is placed in an aeration train as follows:

No. 1 20 ml $\frac{N}{20}$ ceric Sulphate in $\frac{N}{1}$ sulphuric acid.

No. 2 20 ml 20% w/v Silver sulphate in $\frac{N}{1}$ sulphuric acid.

No. 3 20 ml. $\frac{N}{1}$ Sodium Hydroxide solution.

No. 4 Sample.

No. 5 1 ml. $\frac{N}{5}$ sodium hydroxide solution.

No. 6 1 ml. $\frac{N}{5}$ sodium hydroxide solution.

3. Oxygen free nitrogen is bubbled through the train at a flow of 750 mls/minute for one hour.

4. After this time the sample is diluted to 50 mls. as before and 1 ml. samples used to measure the thiocyanate concentration.

5. The 1 ml. samples of sodium hydroxide act as an alkali trap for cyanide and the measurements are carried out on them directly by the method of Aldridge. The sum of their concentrations gives the cyanide concentration in the original sample of plasma, i.e. in 5 mls. plasma.

The cyanide estimation in urine requires a slight modification of the aeration technique, as follows:-

Tube Nos. 1-3 as before.

No. 4 20 ml. aliquot with addition of 4 ml. 4N sulphuric acid.

No. 5 repeat of ceric sulphate solution.

No. 6 repeat of silver sulphate solution.

No. 7 20 mls. $\frac{N}{10}$ sulphuric acid.

Nos. 8 and 9 alkali trap.

The aeration time is 2 hours. The alkali trap is changed after 1 hour, and the cyanide content is distributed in 4 samples of 1 ml. sodium hydroxide.

After aeration dilution of the 20 ml. aliquot to 200 mls. is carried out as before and 1 ml. samples are taken for thiocyanate analysis.

Serum Thiocyanate Concentrations in Tobacco Amblyopia.

(a) Prior to Treatment.

Serum thiocyanate determinations by Bowler's method (1944) were made on three groups of subjects. Group I consisted of 40 patients suffering from tobacco amblyopia, diagnosed according to the criteria of Heaton et al (1950). In group II were 37 non-amblyopic smoking subjects drawn from a hospital population. Group III contained 60 non-smoking hospital patients. Those in group II and III were elderly patients attending hospital with conditions unrelated

to tobacco amblyopia. The age composition of all three groups was similar. Patients in the three groups were classified at each of three levels of serum thiocyanate concentration as shown in the figure. Sixty one per cent of the non-smokers had a thiocyanate concentration of 0.5 mg. per cent or less, only 6.5 per cent having a concentration of 1.5 mg. per cent or more. In contrast, a serum thiocyanate concentration of 0.5 mg. per cent or less was found in 38 per cent of non-amblyopic smokers, whereas 32 per cent had concentrations of 1.5 mg. per cent or more. The distribution of serum thiocyanate concentrations in these two groups is significantly different. (x^2 = 11.76, n = 2, p 0.01) confirming the findings of Ston (1957) that serum thiocyanate concentrations are higher in smoking than in non-smoking subjects (Figure 4.2).

As outlined, the mean tobacco consumption of the group of patients suffering from tobacco amblyopia was higher than that of the non-amblyopic smokers. In spite of this, the group of amblyopic patients in general showed much lower serum thiocyanate than did the group of non-amblyopic smokers, the distribution of thiocyanate

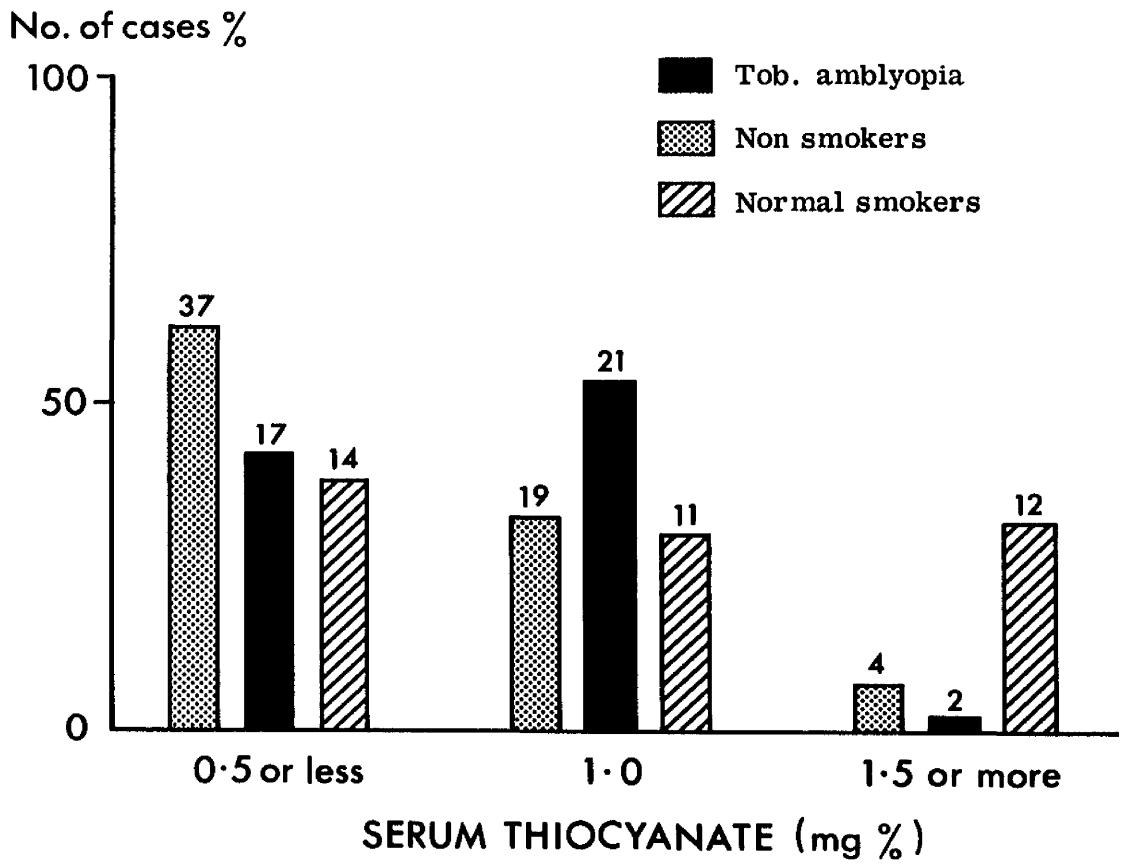


Figure 4. 2. Histogram for serum thiocyanate concentrations comparing subjects with tobacco amblyopia, non-amblyopic smokers and non-smokers.

concentrations being significantly different in the two groups ($x^2 = 10.47$, $n = 2$, $p < 0.01$).

Somewhat surprisingly, in spite of their heavy consumption of tobacco, the distribution of thiocyanate concentrations in this group of amblyopic patients resembled that of the non-smokers and in fact the distribution of thiocyanate concentrations in these two groups was not significantly different ($x^2 = 3.5$, $n = 1$, $p > 0.05$). (See appendix B,3).

These relatively reduced concentrations of serum thiocyanate in tobacco amblyopes as compared with healthy smokers, suggest that if cyanide derived from tobacco smoke is indeed a factor of aetiological importance in the development of tobacco amblyopia, the biochemical defect in these cases may be a failure of conversion of cyanide to thiocyanate, its main detoxification product, as suggested by Smith and Duckett (1965).

B. <u>During Treatment with Hydroxocobalamin</u>

Serum thiocyanate estimations were repeated after

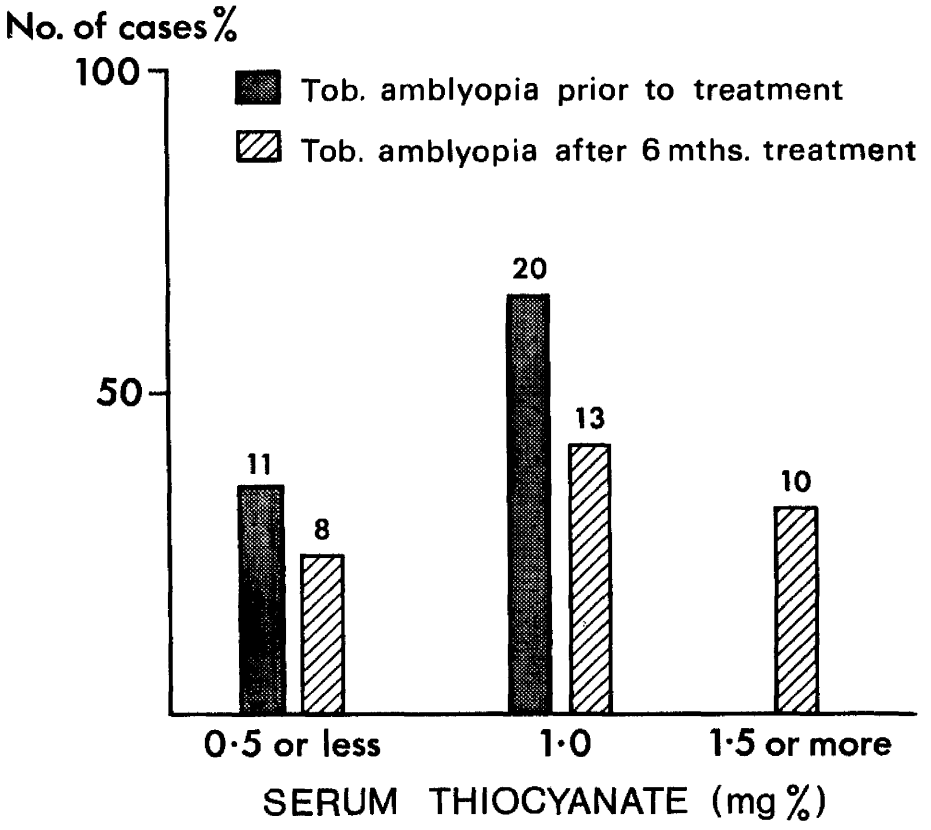


Figure 4, 3. Histogram showing serum thiocyanate concentrations comparing patients suffering from tobacco amblyopia prior to and after 6 months treatment with hydroxocobalamin.

six months treatment with hydroxocobalamin in the group of patients with tobacco amblyopia. Of the 40 patients with tobacco amblyopia who had serum thiocyanate estimations carried out by Bowler's method described above, only 31 were available for repeat analysis after 6 months. The reasons for this included replacement by Aldridge's method for Bowler's method, default, and death of a patient. Details of the treatment are given in the appropriate section.

Whereas 11 of the amblyopes had a serum thiocyanate level of 0.5 mgm% or less, 20 had a level of 1.0 mgm% and none a level of 1.5 mgm% or greater; after 6 months treatment 8 subjects had a level of 0.5 mgm% or less, 13 a level of 1.0 mgm% and 10 a level of 1.5 mgm% or greater. There is a significant alteration in the distribution of thiocyanate levels in the treated group ($x^2 = 10.02$, $n = 2$, $p = 0.01$). No significant difference in distribution is detected when the treated group is compared with the non-amblyopic smoking group of the previous section ($x^2 = 1.57$, $n = 2$, $p = 0.5$).

Plasma Cyanide and Thiocyanate Concentrations in Untreated Tobacco Amblyopia.

Wilson and Matthews (1966) showed that a negative relationship existed between the plasma cyanide and serum vitamin B12 concentrations in healthy smokers and non-smokers. Plasma cyanide concentrations were estimated in 11 patients suffering from tobacco amblyopia by the method described above and the serum vitamin B12 concentration was estimated after the method of Ross (1952). See Table 4, 1 for results.

Of these patients the mean cyanide concentration was 0.065 $\pm$ 0.024 micro moles/100 mls. This value is considerably higher than the value of 0.022 micro moles/100 mls. found by Wilson and Matthews (1966) in non-amblyopic smokers by a similar method of analysis. When the plasma cyanide and serum vitamin B12 were compared a negative correlation was obtained, and this proved to fall just within the significant level ($r = -0.555$, $n = 9$, $0.05 < p < 0.1$). Although not a very significant correlation, possibly due to the small number in the sample, the general

Tobacco Intake ozs/wk.	Serum Vit. B12 pg/ml.	Plasma CN umoles/100mls.	Plasma SCN umoles/100ml.	Urinary SCN u/mole/100mls.	Renal thiocyanate Untreated	Clearance after 6/52 therapy
3.0	520	0.08	3.9	-		
1.5	536	0.07	0.86	-		
2.5	316	0.106	6.0	4.7	0.65	
2.0	352	-	1.2	1.7	1.087	
2.0	250	0.07	2.5	-		
2.0	360	0.046	1.86	0.8	0.672	0.843
3.0	160	0.074	1.85	1.0	0.35	
2.0	324	-	1.2	1.3	0.948	1.49
3.0	350	0.04	8.4	1.9	0.143	0.896
3.0	350	0.04	11.7	1.93	0.103	
5.0	490	0.07	3.0	1.15	0.442	0.884
1.0	554	0.024	0.6	1.15	1.25	1.39
2.0	132	0.102	6.45	4.56	0.368	
0.5	84	-	-	2.13		
2.0	110	-	-	1.7		
4.0	435	-	-	2.74		
Mean values		0.065	3.81	2.06	0.601	1.100

trend does support the view that high plasma cyanide concentrations in the presence of reduced serum vitamin B12 concentrations could be associated with neurological lesions.

On comparing the plasma cyanide concentration with the consumption of tobacco (the presumed source of cyanide) in these patients a positive correlation was obtained which failed to be significant ($r = 0.344$; $n = 7$; $p > 0.1$).

The mean plasma thiocyanate concentrations of 13 untreated tobacco amblyopes was found to be 3.81 ± 3.306 micro moles/100 mls. This value is lower than that obtained, by a similar method of analysis, by Wilson and Matthews (1966) who found a mean concentration of 11.5 micro moles/100 mls. in 12 non-amblyopic smokers. These authors further described a significant positive correlation between the plasma cyanide and plasma thiocyanate concentrations. On comparing these two factors in the present group, the correlation obtained, although positive was not significant ($r = +0.351$; $n = 7$; $p > 0.1$).

Urinary Thiocyanate Concentration in Untreated Tobacco Amblyopia.

As described above, a rise in the serum thiocyanate concentration occurs on treatment with hydroxocobalamin for tobacco amblyopia. If the renal clearance of thiocyanate remained at the value of 1.33 ml. per minute, found by Stoa (1957), then the rise in blood thiocyanate may be reflected by a rise in urinary excretion. Thomson (1902) found that the toxicity of the urine in tobacco amblyopes, treated by abstinence from tobacco, reached a peak 7-14 days after the commencement of treatment and was accompanied by a diuresis. This increase in toxicity was taken by him to indicate the body getting rid of poisonous substances. The renal clearance of thiocyanate was found by Thomas (1949) and Stoa (1957) to depend on urine volume and the renal excretion of chloride, as the thiocyanate ion behaves as a pseudo halogen. Wokes and Ellis (1966) found that the diurnal rhythm of renal thiocyanate clearance paralleled that of the total nitrogen clearance.

(a) The relationship between the urinary thiocyanate excretion over 24 hours and the serum vitamin B12 concentration was shown by Linnell et al (1968) to be negative in non-amblyopic smoking subjects. The values for urinary thiocyanate estimated as outlined above and the serum vitamin B12 concentration after the method of Ross (1952) were compared. They yielded a negative correlation which showed a significant trend ($r = -0.479$; $n = 2$; $0.05 < p < 0.1$).

(b) The renal excretion of thiocyanate was followed over a period of 6 weeks in 14 patients whilst on hydroxocobalamin therapy. The results are expressed graphically in Figure 4, 4. On the same graph is shown the mean alteration in urine volume for the same patients.

TIME IN WEEKS	0	1	2	4	6
MEAN URINARY THIOCYANATE IN MICRO MOLES / 24 HOURS	27.3 (100%)	30.44 (111.5%)	37.6 (137.7%)	33.29 (121.9%)	29.92 (109.5%)
MEAN URINE VOLUME IN MLS.	1464 (100%)	1850 (126.3%)	1778 (121.4%)	1536 (104.9%)	1301 (88.8%)

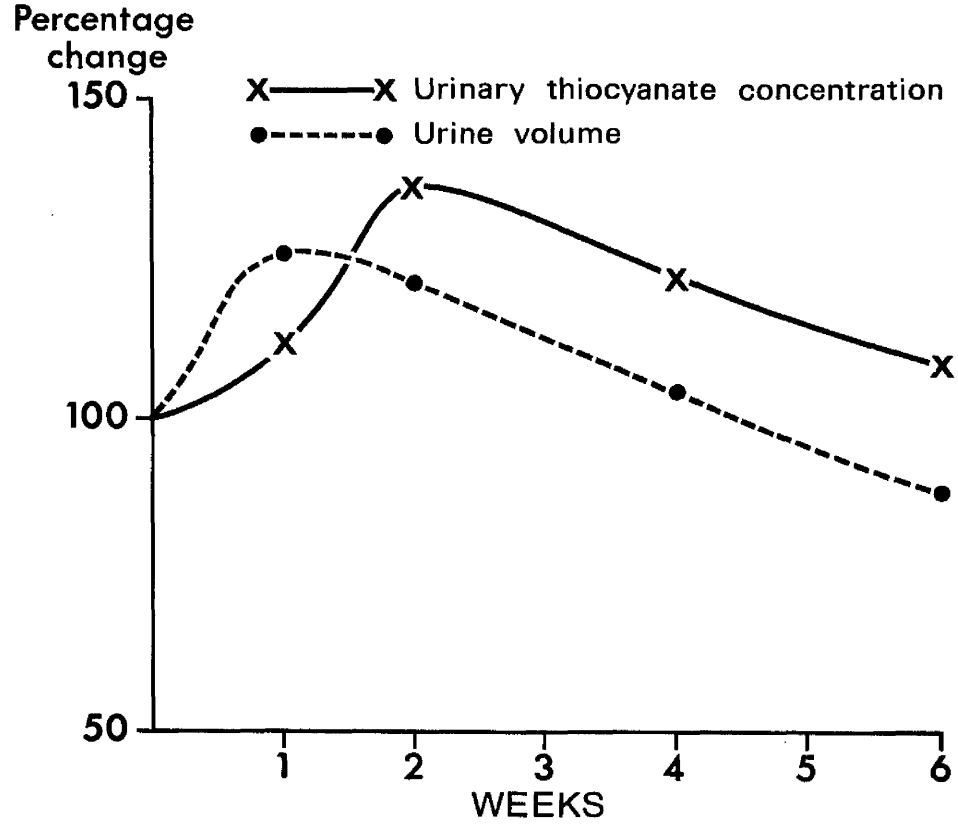


Figure 4, 4. -Alteration in urinary thiocyanate concentration and urine volume in tobacco amblyopia on treatment with hydroxocobalamin.

There was a rise in the urinary excretion of thiocyanate on treatment with hydroxocobalamin, which reached a peak at 2 weeks, showing a 40% increase, before slowly declining. A similar rise was noted in the urinary volume.

(c) Stoa (1957) examined the renal thiocyanate clearance in healthy smokers and non-smokers. He found an average clearance of 1.33 ml. per minute, with a range of 0.14 - 3.53 mls. per minute. There was no correlation between the thiocyanate concentration in the blood and the renal clearance for thiocyanate. In the present group of patients a significant negative correlation was found between these two factors ($r = -0.762$; $n = 8$; $0.001 < p < 0.01$) (Figure 4,5). After six weeks therapy with hydroxocobalamin the correlation showed a trend to becoming positive ($r = +0.770$; $n = 3$; $p > 0.1$). The average clearance of thiocyanate rose from 0.601 to 1.1 mls. per minute on this therapy after six weeks.

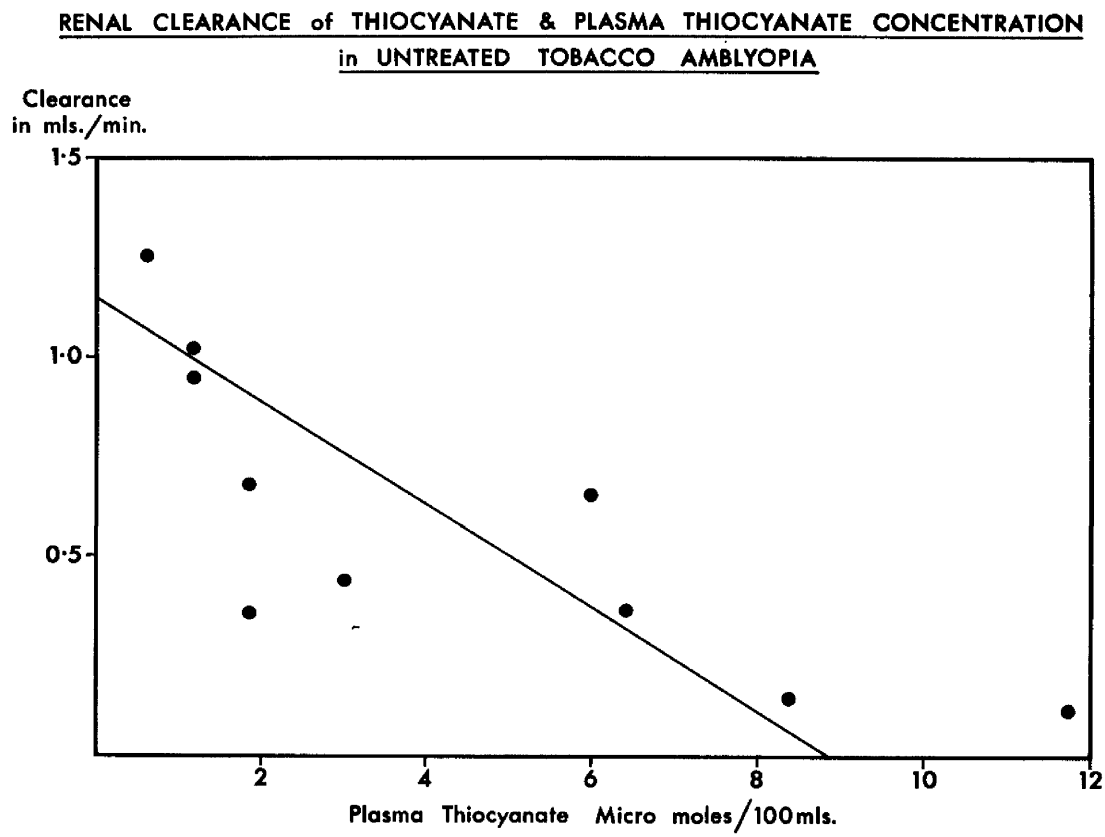


Figure 4. 5. Relationship between renal clearance of thiocyanate and plasma thiocyanate concentration in untreated tobacco amblyopia.

Thus the renal clearance of thiocyanate varies inversely with the plasma thiocyanate concentration, low thiocyanate clearance rates being associated with high plasma thiocyanate concentrations. A comparison was made between renal thiocyanate clearance and tobacco consumption. The date for 9 patients was available; a negative correlation was obtained which was just significant ($r = -0.681$, $n = 7$, $0.02<p<0.05$).

Thus low renal clearances of thiocyanate are associated with high tobacco consumptions and high plasma thiocyanate concentrations and high renal clearances of thiocyanate are associated with low tobacco consumptions and low plasma thiocyanate concentrations.

CHAPTER V.

RESPONSE TO THERAPY

Abstinence from tobacco has been considered necessary, important, or desirable in the successful treatment of tobacco amblyopia (Hutchinson 1873, Webster 1880, Hartridge 1886, Chisholm 1890, Fairley 1901, Lyle 1905, Carroll 1935, Hembresin and Schepens 1946, Hedges 1957, Doggart 1959). For many years this belief was the keystone of treatment, till Carroll (1937) showed that abstinence was not necessary. The effect of pharmacological preparations has been investigated by authors to ascertain which, if any, accelerated return of visual function.

The earliest and most popular drug used was strychnine. Injections of this drug were considered to hasten recovery by Chisholm (1878); combined therapy with strychnine and potassium iodide was used by Lautenbach (1898) and Findlay (1901). Hartridge (1886) in a controlled series, discontinued smoking in all patients, administered strychnine to half, and a placebo to the other

half. The rate of improvement in either group was equally good.

Vasodilating nitrites were used by Powers (1886) with beneficial results. The success with the use of sodium nitrite found by Gordee and Harrington (1935) was not confirmed by Carroll (1937). Bonnefon (1931), Gragg (1936), Orr (1934) and Duggan (1937) felt that visual improvement was hastened by administering acetyl choline and that this drug was superior to sodium nitrite.

Carroll (1937-36) found that patients on adequate diets made a partial or complete recovery, in spite of continued use of tobacco and/or alcohol. He claimed his results were as good as any previous series in which the patients abstained from tobacco. Carroll further showed that large doses of vitamin B complex and a well balanced diet were essential for a rapid return of vision. Some success was obtained by Johnson (1939) by the administration of vitamin B1 (Thiamine).

Heaton et al (1950), on finding reduced

concentrations of vitamin B12 in the blood of patients suffering from tobacco amblyopia, treated their patients successfully with parenteral cyanocobalamin whilst allowing continued smoking. Their patients recovered vision more quickly than would have been expected on treatment with vitamin B complex. The dose schedule was 100µg twice weekly, where anaemia was absent, 100 µg daily for fourteen days followed by 100 µg twice weekly, where anaemia was present. Treatment was discontinued after six months if there was no evidence of pernicious anaemia. Quatermass (1958) achieved some success with parenteral cyanocobalamin in the amblyopia found in a smoking alcoholic.

It had been shown that commercial preparations of cyanocobalamin contained traces of other vitamin B12 analogues (Baxter et al 1953), notably of hydroxocobalamin. This, reasoned Smith (1961) had brought about the cure in the patients reported by Heaton et al (1958). As the result of a personal communication this author is assured that the commercial preparations of cyanocobalamin and hydroxocobalamin available in Great Britain today do not now contain other vitamin B12 analogues as impurities. (Snell 1967).

Treatment of Tobacco Amblyopia Patients.

The patients in the present study were treated with preparations of vitamin B12 manufactured by Glaxo Laboratories Ltd. They were requested not to alter their smoking habit. In general, the patients received their first fourteen days treatment as in-patients in hospital. They were reviewed, as near as possible, at monthly intervals. Adjustments to therapy were made from time to time depending on the therapeutic response. The follow up period varied from five months to thirty five months with a mean at nineteen months.

The progress of each patient at the end of the follow up period was assessed visually and graded by a series of symbols:-

0 = no change

- = Worsening in visual acuity

+ = Improvement in visual acuity to 6/18 Snellen or 35% on the percentage acuity scale.

++ = Improvement in visual acuity to 6/12 Snellen or 50% on the percentage acuity scale.

+++ = Improvement in visual acuity to 6/9 Snellen or 65% on the percentage acuity scale.

Taking a visual result of 6/12 or better at the end of the follow up period, as a good result and 6/18 or worse as a poor result, there were 40 with a good result and 15 with a poor result. The remaining patients (10 in number) were lost from the survey by death and default. Of the 15 patients in the poor group, 4 were undergoing a recurrence of the disease and it may be possible that these patients had not fully recovered from their previous attack of the disease. All 6 patients who had experienced a previous attack of the disease, had been treated by abstinence from tobacco not less than 7 years before, and in all the disease recurred after a variable interval on resuming smoking.

Duration of Symptoms and Visual Outcome.

OUTCOME	+++	++	+	0	-
NUMBER IN GROUP	30	10	6	1	8
MEAN DURATION OF SYMPTOMS	4.9 months	8.6 months	9.3 months	3 months	11.4 months

Thus 40 patients (72.2%) whose mean duration of symptoms, before seeking advice, was 5.45 months, had their vision restored to 6/12 (Snellen) or better during the mean follow up period of 19 months and the remaining 15 (27.8%) (mean duration of symptoms 9.6 months) showed little or no improvement. There is a significant difference between these groups ($t = 2.07$; $u = 53$; $p<0.05$). Thus it would appear that the duration of symptoms influenced the outcome.

Rate of Visual Improvement.

The 30 patients who had a visual improvement to 6/9 (Snellen) or better were examined for the rate of visual improvement. This group consisted of 26 patients who received hydroxocobalamin while continuing to smoke, 3 patients who were treated by abstinence from tobacco, and one patient who failed to respond to hydroxocobalamin and in whom visual improvement only occurred when smoking was stopped in addition to receiving hydroxocobalamin (Patient No. 52). This patient is excluded from this analysis.

Of the 26 patients treated with hydroxocobalamin only, the mean period of treatment over which the visual improvement occurred was 6.82 ± 4.49 months. The improvement in vision was calculated from the ratio

$$\frac{\text{visual acuity\%}}{\text{initial visual acuity\%}}$$

The mean improvement in visual acuity per month of the right eye of the 26 patients was 1.75 ± 2.38 units.

Of the 3 patients treated by abstinence from smoking the mean period required to produce a visual improvement to the same standard was 3.0 months, and the mean improvement in visual acuity per month of the right eye was 1.38 ± 0.34 units. The rate of visual improvement was thus equally good by either method of treatment ($t = 0.264$; $u = 27$; $p > 0.1$).

Comparison of Therapeutic Response to Cyanocobalamin and Hydroxocobalamin.

Initially patients were placed on therapy alternately with cyanocobalamin and hydroxocobalamin. By analysing the first thirteen patients of whom seven

were receiving cyanocobalamin, and six, hydroxocobalamin, it was clear that hydroxocobalamin was the superior treatment. The dosage used was usually 1000 µg daily for two weeks followed by 1000 µg twice weekly for four weeks, and thereafter 1000 µg at monthly intervals. One patient however, (No.7) received 1000 µg. of cyanocobalamin once weekly for one month followed by 1000 µg fortnightly while another patient (No. 1) received 1000 µg. of cyanocobalamin daily for one week followed by 500 µg. fortnightly.

GROUP 1 (Treated with Cyanocobalamin)						
Patient Number	Tobacco intake (ozs/week)	Duration of Visual Symptoms (months)	Initial Visual Acuity	Initial 100 Hue Score	Visual Acuity at 5 months	100 Hue Score at 5 months
1	1.0	9	2/60	-	2/60	-
7	2.0	2	2/36	-	6/60	758
18	2.5	24	1/60	904	2/60	944
38	3.0	12	6/18	1068	6/12	1057
41	2.0	2	6/36	-	6/6	-
48	3.0	2	1/60	1120	3/60	1221
59	5.5	2	6/60	623	2/60	531
GROUP 2 (Treated with Hydroxocobalamin)						
3	4.5	4	6/18	339	6/6	177
11	7.0	5	6/60	996	6/24	630
12	4.0	12	3/60	600	6/9	327
22	3.0	4	6/24	824	6/12	505
54	1.5	3	6/60	676	6/9	256
58	3.0	9	6/36	625	6/18	536

Details of the patients treated are set out in Table 5, 1. All but one were pipe smoking elderly males, the exception was one who smoked only cigarettes. All had impairment of central vision and of colour vision at the start of the trial. The mean age of the patients in each group was similar (Group 1 65.7 years; group 2 61.3 years) although group 1 contained some patients who were older than any in group 2. The mean tobacco consumption in group 2 was higher then in group 1 (3.0 ozs. per week as compared with 2.7 ozs. per week). The mean length of visual history was similar in each group (group 1; 7.6 months; group 2 6.2 months). The slightly higher figure for group 1 was due to the presence in this group of one patient with a very long history of visual failure (two years).

To assess the results of treatment, the visual acuity on the Snellen test type at 6 metres under standard conditions of illumination was expressed as a percentage taking 6/6 as 100% (Ridley 1959). The improvement in vision per month was calculated as shown on page I48.

The mean improvement in visual acuity per month

of the poorer eye of each case in the two groups is shown in Figure 5, 1, where it can be seen that both the rate and magnitude of the improvement in vision of those cases treated with hydroxocobalamin is greater than of those treated with cyanocobalamin. The recovery of vision in the two groups is equivalent to an improvement from 6/60 to 6/9 in the case of those treated with hydroxocobalamin and from 6/60 to 6/36 partly in those treated with cyanocobalamin.

There is a significant statistical difference between the two therapies. [The mean improvement in vision over five months for those cases treated with hydroxoco: :balamin = 5.54 $\pm$ 4.4 units. Mean improvement in vision over five months for the cases treated with cyanocobalamin = 1.91 $\pm$ 1.64 units. ($t = 2.39$; $n = 2$; $p = < 0.05$)]

Measurements of colour discrimination were also used to assess the progress of the condition in the patients described above. The initial and final error scores with this test for each patient are shown in Table 5,1 where it can be seen that prior to the start of treatment, the error score was grossly abnormal in all

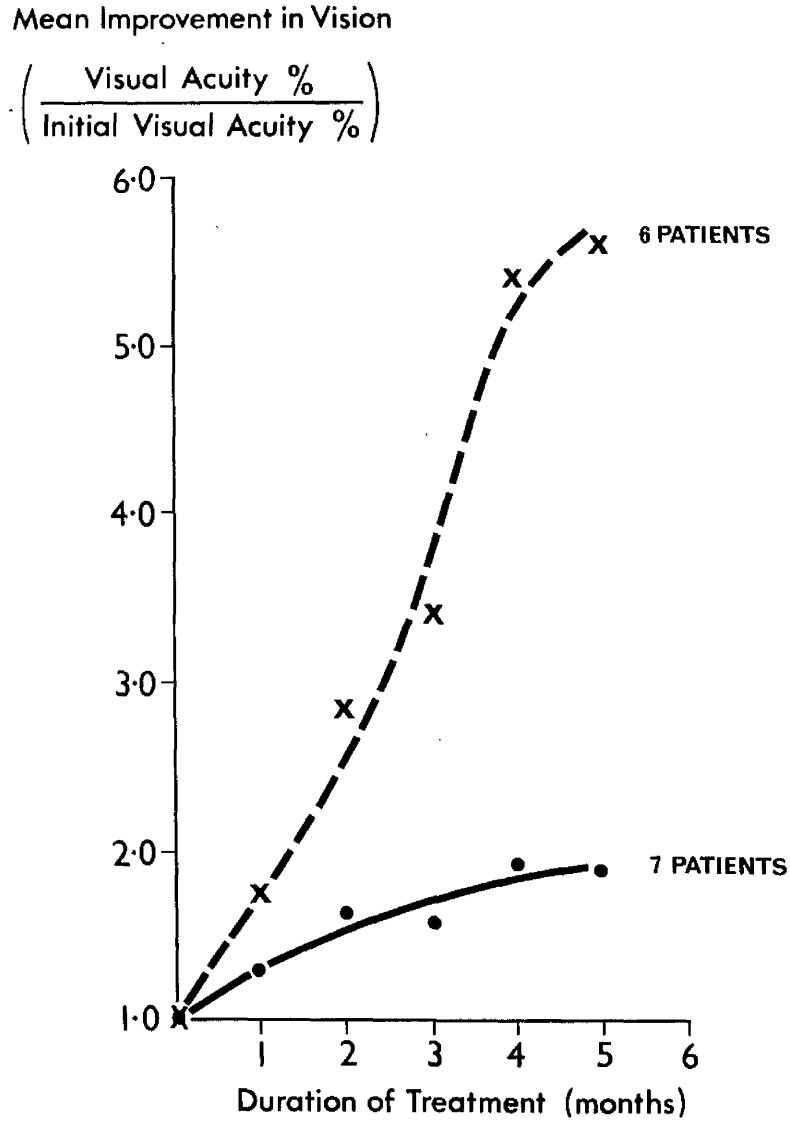


Figure 5. 1. Comparison between the rate of visual improvement per month in patients with tobacco amblyopia treated with parenteral hydroxocobalamin (X--X) and cyanocobalamin (O-O-). The rate of visual improvement and the maximum improvement occurring is significantly better in those patients treated with hydroxocobalamin.

patients tested. In figure 5, 2, the mean error score for those patients on treatment with hydroxocobalamin has been compared at monthly intervals with those treated with cyanocobalamin. In each case the figure for one eye only has been taken, the eye selected being that with the poorer colour discrimination at the start of the trial. Again, it can be seen that there is a greater improvement in colour discrimination in response to treatment with hydroxocobalamin than to cyanocobalamin.

The marked difference in the response of tobacco amblyopia to hydroxocobalamin as compared with cyanocobalamin is borne out by consideration of the subsequent behaviour of those cases initially treated with cyanocobalamin who are now receiving hydroxocobalamin. In four cases (patients Nos. 1, 7, 38 and 41) the visual acuity improved to 6/9 after four months of treatment with hydroxocobalamin. (A published account of the findings of this comparison is to be found in the appendix B1).

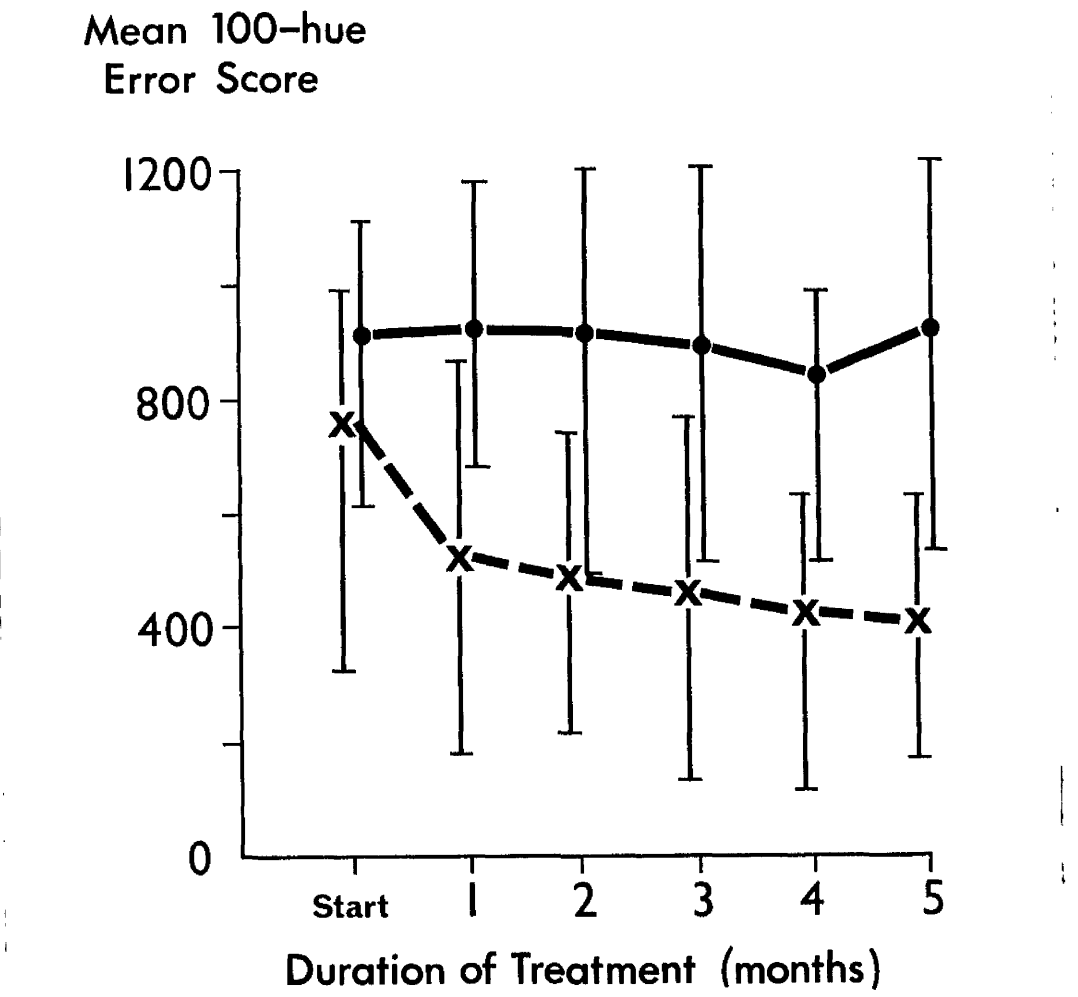


Figure 5. 2. Mean improvement in colour discrimination in patients with tobacco amblyopia treated with hydroxocobalamin (X--X) as compared with cyanocobalamin (O--O). Improvement in colour vision (a reduction in 100 Hue error score) occurs among patients treated with hydroxocobalamin but not among those treated with cyanocobalamin. Vertical bars indicate the range of scores.

Following on the comparison of the rate of improvement following therapy with cyanocobalamin and hydroxocobalamin, and the favourable outcome for hydroxocobalamin, all subsequent patients have been placed on therapy with hydroxocobalamin.

Disproportion between rate of visual recovery and colour sense recovery.

Fig. 5, 3 is a graphic representation of the improvement in visual acuity and Farnsworth 100 Hue error score on treatment with hydroxocobalamin. The patient is a pipe-smoking male aged 69 years who had been smoking 4-5 ozs. of pipe tobacco weekly and had a daily intake of alcohol. His visual symptoms were for five months only. Corrected visual acuity in the right eye was 6/18 and in the left eye 6/12. Centro-caecal defects to colour were found in both visual fields and the Farnsworth Munsell 100 Hue test error score in the right eye was 339 and in the left 434; serum vitamin B12 was 118 pg/ml. He was placed on treatment with hydroxocobalamin and continued to smoke. After four months treatment the visual acuity had risen

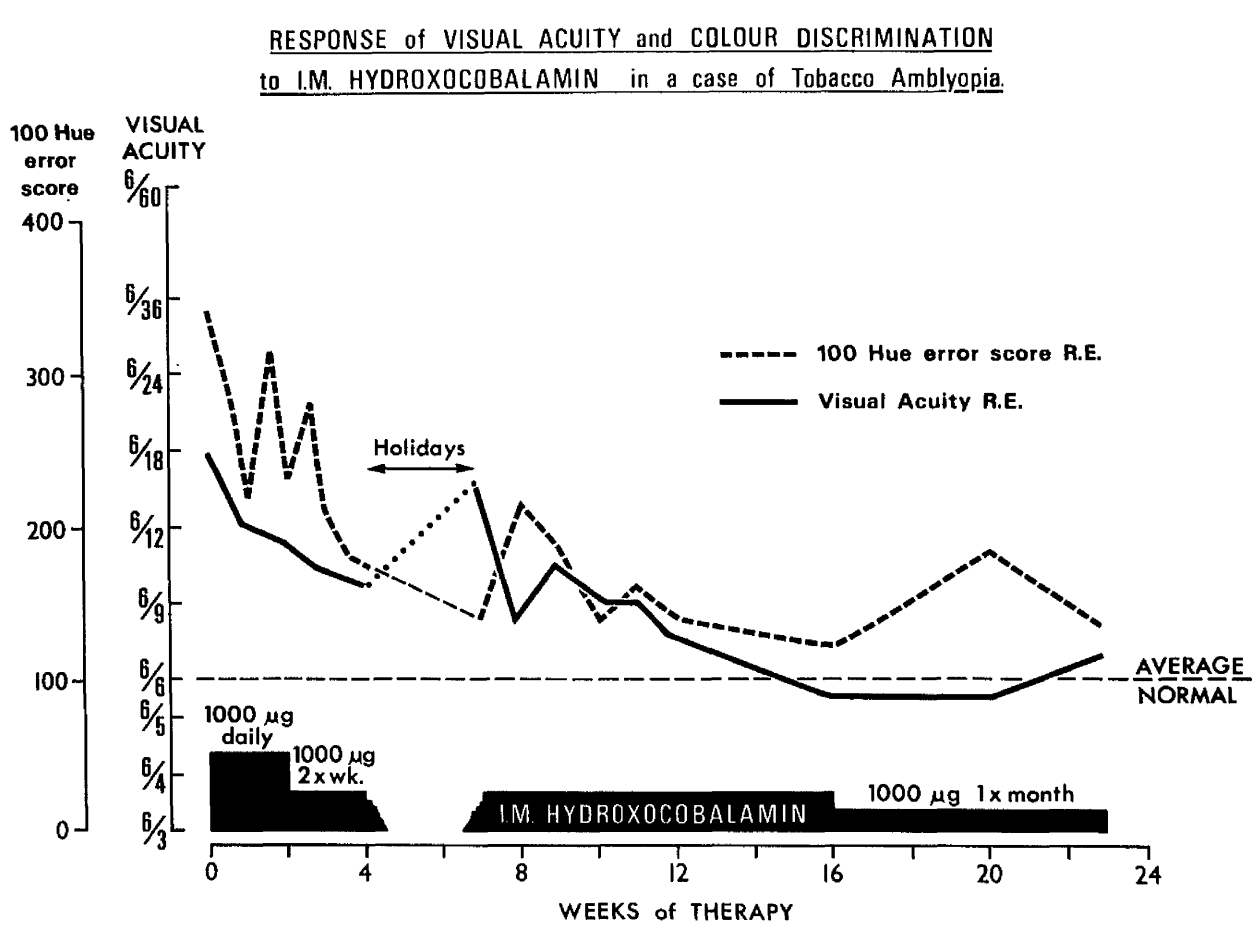


Figure 5, 3. Comparison of visual and colour discrimination improvement with time, on hydroxocobalamin. Patient No.3. It can be seen that improvement in colour vision lags behind improvement in visual acuity.

to 6/9 in the right eye and 6/6 in the left. He has now been on treatment for 22 months. He is currently receiving 1,000 µg of hydroxocobalamin three monthly and his visual acuity is 6/6 right and left, Farnsworth Munsell error score right eye 104, left eye 134.

It is apparent from figure 5, 3 that the improvement in colour discrimination lags behind the improvement in visual acuity, confirming the earlier finding of Riddell (1936).

Rate of Recovery of the Colour Sense.

As there is abundant evidence that estimates of the colour sense provides as sensitive an index of retinal function as does visual acuity, estimations of patients visual improvement were also based on the Farnsworth Munsell 100 Hue test of colour discrimination result. This can be observed by examining the changing profile, or comparing the numerical error score against time on treatment.

(a) An example of change in Farnsworth-Munsell 100 Hue profile is given by following the progress of a 50 years old male who smoked 1½ ozs of pipe tobacco a week and gave a 6 months history of visual failure and trouble with identity of colours. (Patient No.54). The corrected visual acuity of the right eye was 6/60 and of the left eye 6/24. The Farnsworth-Munsell 100 Hue error score for the right eye was 676 and for the left eye 488. Examination of the optic discs showed temporal pallor and there was a centro-caecal field defect in the visual field for each eye. On treatment with hydroxocobalamin the changing profile of the right eye can be followed from Figs. 3, 4,5,6 and 7. With treatment there is shrinkage of the area containing the error.

(b) When the numerical error score is compared with time on treatment a curve is produced. The figures fit an exponential curve of the equation.

$$y = A_e^{-kt} + c$$

$$(y-c) = A_e^{-kt}$$

$$\log_e (y-c) = \log_e A - kt.$$

Name..... Age..... Date...../ ...../

85	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	
43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	
64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	

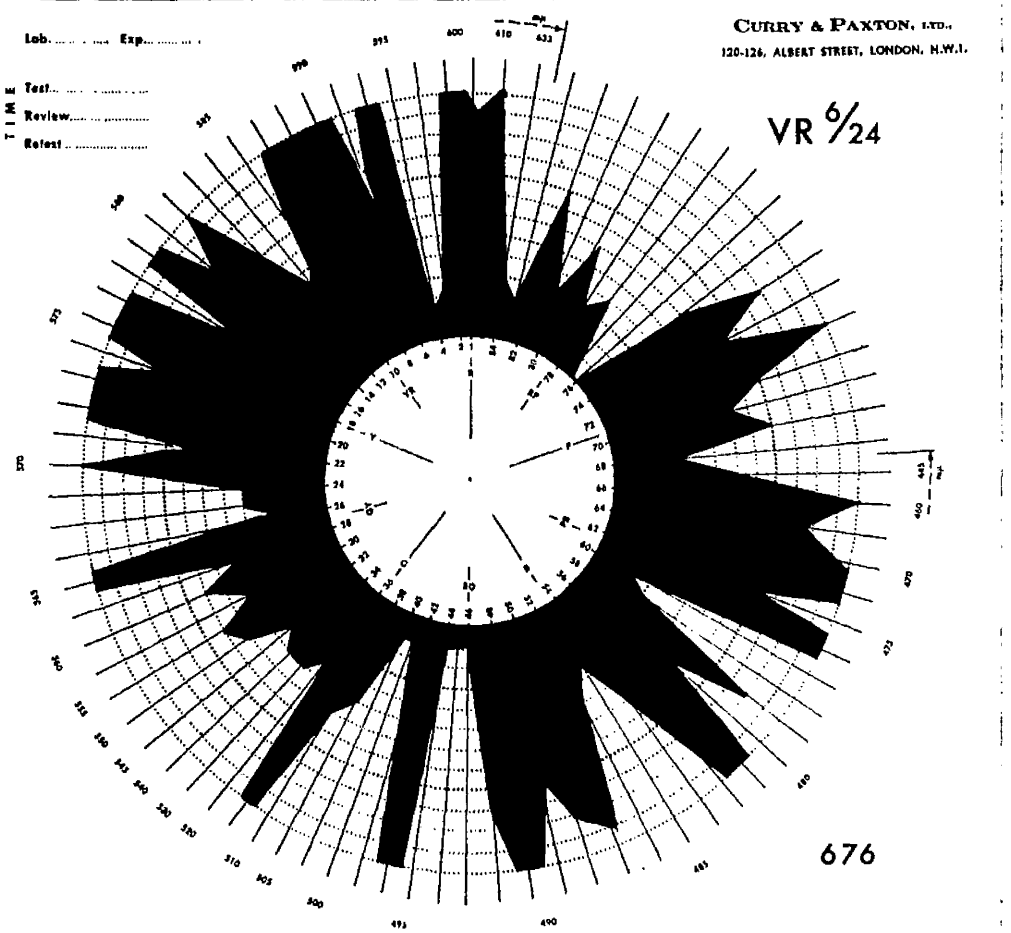


Figure 5. 4. Farnsworth Munsell Hundred Hue test profile in untreated tobacco amblyopia. Patient No. 54.

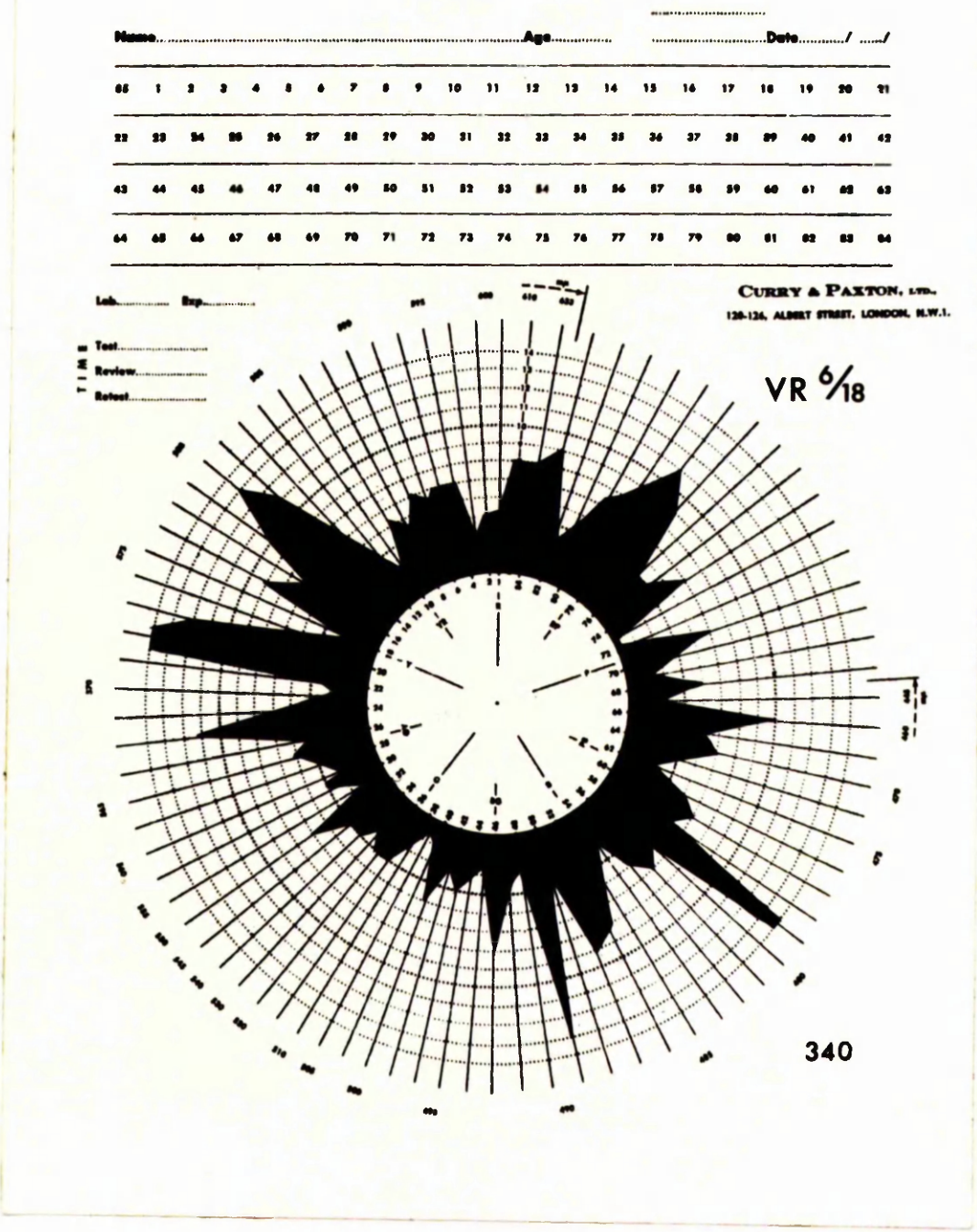


Figure 5, 5. Farnsworth Munsell 100 Hue test profile after 1 month's treatment with hydroxocobalamin for tobacco amblyopia. Patient No. 54.

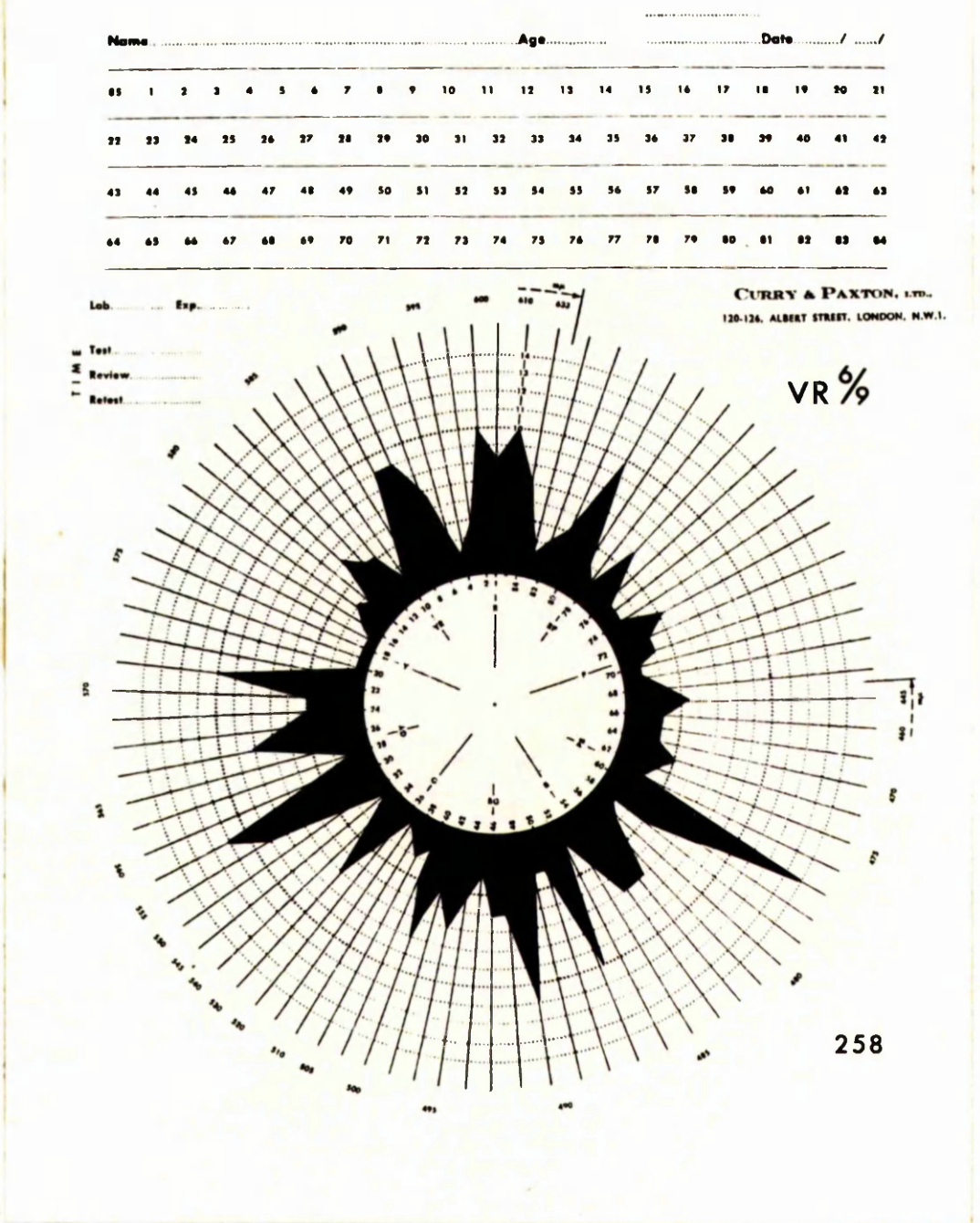


Figure 5, 6. Farnsworth-Munsell 100 Hue test profile after 3 months treatment with hydroxocobalamin for tobacco amblyopia. Patient No. 54.

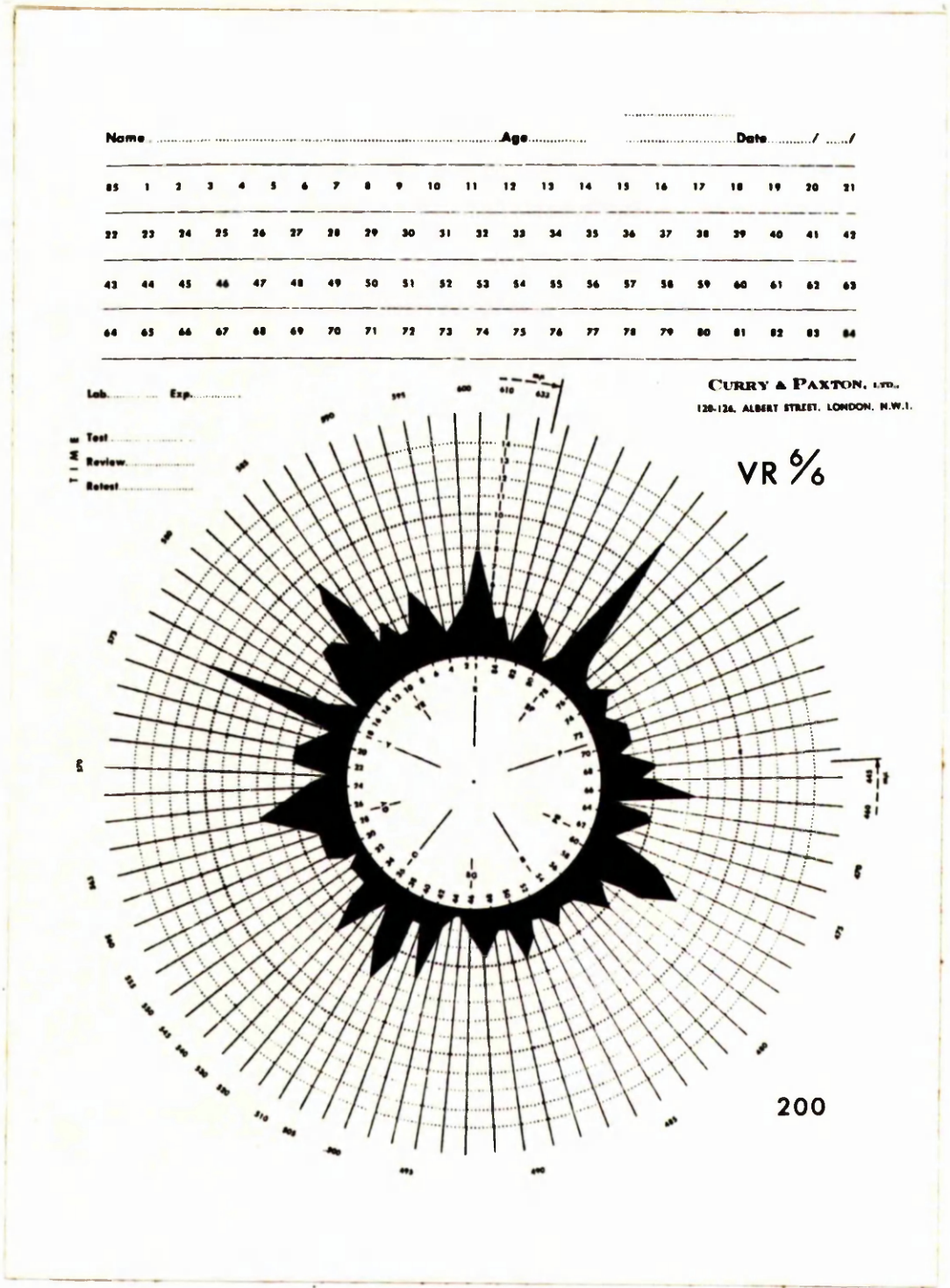


Figure 5, 7. Farnsworth Munsell 100 Hue test profile after 6 months treatment with hydroxocobalamin for tobacco amblyopia. Patient No. 54.

Where y = Raw 100 Hue error score

c = Constant figure which is an individual characteristic of each patient. It is the error score at which the exponential curve would become horizontal. This value is dependent on patient age, ocular disease and some systemic diseases which are known to cause an upset in the colour sense. [Diabetes, (Kinnear 1966), Pernicious anaemia (Adams et al 1967)]

k = Slope of $\log_e (y-c)$ against time, and is an index of the rate of improvement in colour discrimination.

t = Time.

Using the formula the curve now becomes linear -

Use of the KDF9 computer at Glasgow University was made to find the most suitable value for C which gave the "best fit" to the results over the first 18 months of treatment.

An illustrative example is that of a 68 years old male (patient No.37) colour matcher of a cotton mill, who smoked 2½ozs. of pipe tobacco weekly and had a visual disturbance extending over two months. The left eye was known to have poorer vision than the right. The corrected

visual acuity of the right eye was 6/36 and of the left eye 6/60 (Snellen). Centro-caecal scotomas were present in the visual fields of each eye. The Farnsworth Munsell 100 Hue test gave an error of 408 with the right eye and 837 with the left. After 12 months of treatment with hydroxocobalamin the visual acuity of each eye had improved to 6/9 pt. (Snellen) and the Farnsworth Munsell 100 Hue error had fallen to 100 with the right eye and 260 with the left. The improvement in the Farnsworth Munsell 100 Hue error score with time is shown in Fig. 5, 8.

With a c value of 20 and plotting log $(y-c)$ against time a significant linear relationship is obtained. ($r = +0.99$, $n = 9$. $p<0.001$). The slope of the regression line is equivalent to the rate of improvement. This line is shown on fig. 5, 9.

By means of this technique, it is possible to compare the rates of improvement in colour discrimination with treatment in many patients. The following groups of patients were available for comparison:-

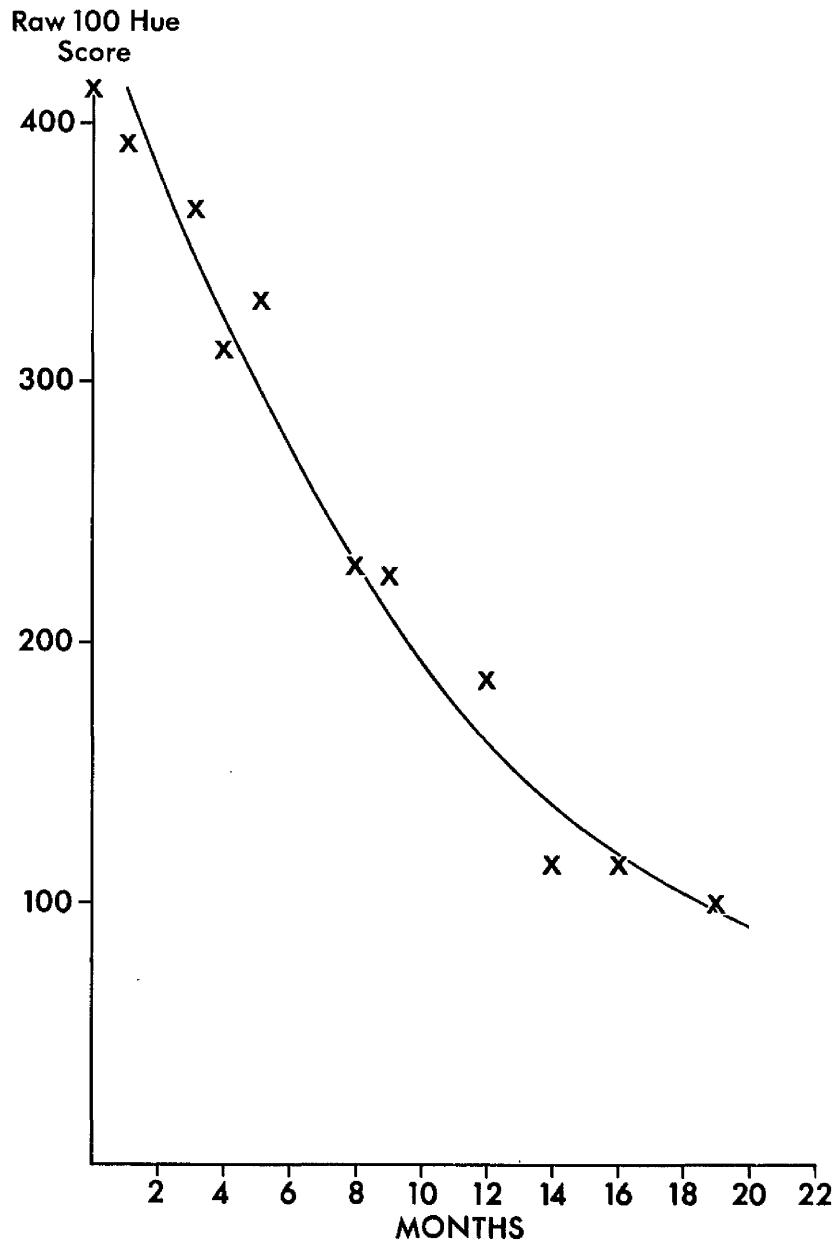


Figure 5. 8. Improvement in colour discrimination with time on hydroxocobalamin. Patient No. 37.

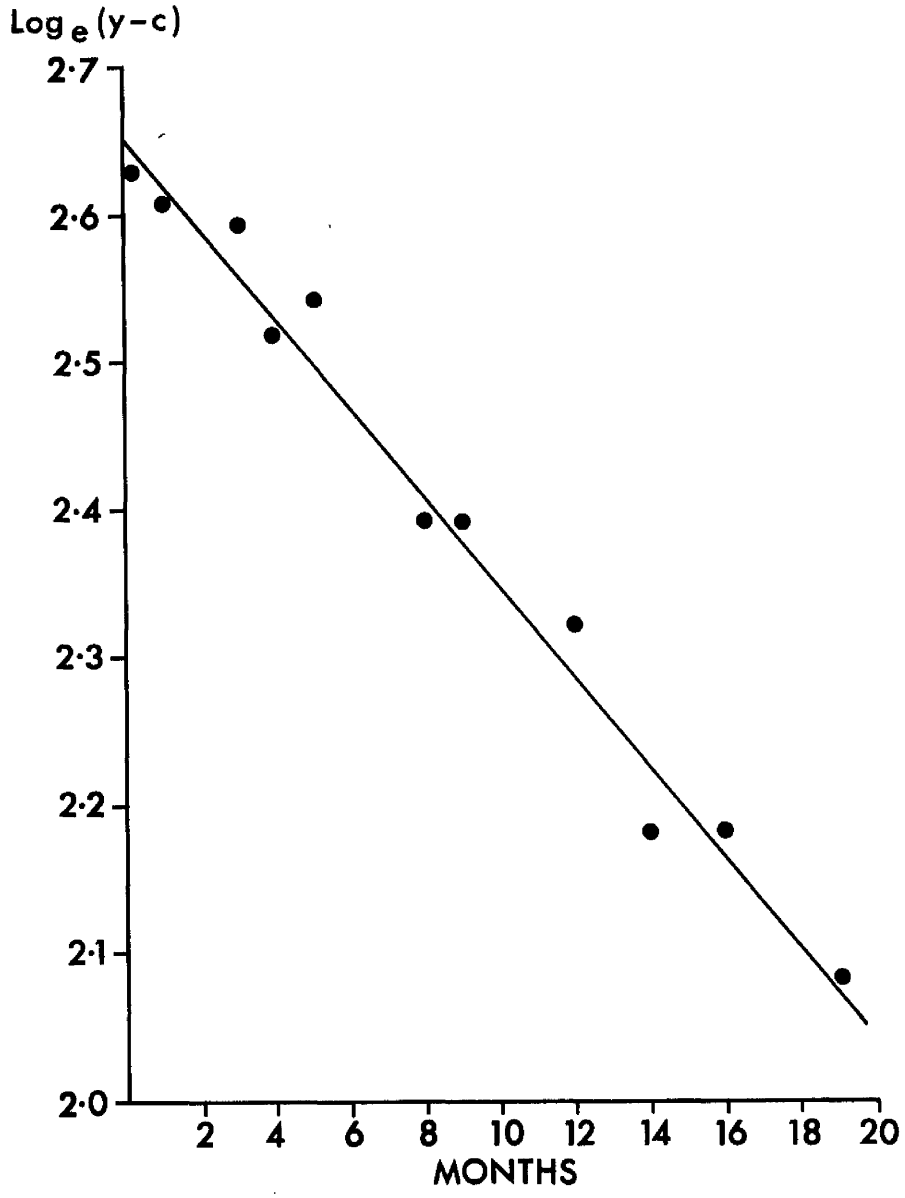


Figure 5. 9. Improvement in colour discrimination with time on hydroxocobalamin. log (y-c) against time. c = 20 ($r = 0.990$). Patient No. 37.

(a) Tobacco amblyopia complicated by pernicious anaemia.

(b) Tobacco amblyopia complicated by malabsorption.

(c) Tobacco amblyopia complicated by diabetes.

(d) Tobacco amblyopia treated with hydroxocobalamin.

(e) Tobacco amblyopia treated by abstinence from tobacco.

The mean values for k (rate of improvement)were obtained for all these groups and are contained below. The value of k is the tangent of the slope angle.

	k	angle
Tobacco amblyopia in presenceof pernicious anaemia	0.195	$2^{0}1'$
Tobacco amblyopia in presence of pre pernicious anaemia	0.378	$20^{0}42'$
Tobacco amblyopia in presence of diabetes	0.564	$29^{0}18'$

These three groups all received the same therapy namely hydroxocobalamin 1000 µg daily for 14 days followed by 1000 µg thrice weekly. Thus the rate of improvement in colour sense was poorest in the group complicated by pernicious anaemia. There is insufficient data to observe whether this could be improved on, by giving a larger dose

of hydroxocobalamin.	K	Angle
Uncomplicated tobacco amblyopia treated with hydroxocobalamin	0.284	$15^{o}48'$
Uncomplicated tobacco amblyopia treated by abstinence from tobacco	0.302	$16^{o}43'$

There is little difference in these two groups, the rate of improvement in the colour sense being equally good by either method of therapy.

CHAPTER VI.

I. LEBER'S HEREDITARY OPTIC ATROPHY

Leber's hereditary optic atrophy is a relatively rare condition characterised by acute or subacute failure of central vision presenting as a retrobulbar neuritis or optic atrophy, typically in young males in their late teens, or early twenties. It is uncommon for only one eye to be affected and equally uncommon for the condition to resolve completely, though examples of complete or nearly complete recovery have been described. Many patients with Leber's hereditary optic atrophy are registered as blind persons as the central scotoma is large and dense in each eye and account for 0.56% of blind registrations (Marshall and Seiler 1942).

Leber (1871) considered that the age of onset was most common between 13 and 28 years. Aeneman (1956) found that the disease began between 20 and 30 years in 47.8% of his series. Several authors have stated that the age of onset in succeeding generations becomes progressively

younger - so called anticipation (Nettleship 1909, Waardenburg 1923).

Assemen (1950) found that the disease invariably began with an acute reduction of vision, which was followed by a period of slow progression which could last for several months. The disease nearly always attacked both eyes, sometimes simultaneously, sometimes with an interval which varied from weeks to months before the second eye was affected. The acute diminution of vision may lead to complete blindness or sufficient impairment to warrant blind certification. In a minority, the condition improves spontaneously after a variable interval, with clearing of the centre of the scotoma leaving a ring scotoma. Generally, however, the patient is left with a dense permanent scotoma affecting the fixation point in each eye. In a study of the dyschromatopsia which accompanies this condition, Francois et al (1961) concluded that the colour defect was characteristically to be found in the red-green areas of the spectrum which served to differentiate Leber's hereditary optic atrophy from the dominantly

inherited optic atrophy in which the yellow-blue regions were affected.

Ophthalmoscopy in the acute phase may reveal a normal optic disc or a disc showing the oedema and hyperaemia of papillitis. The fundus picture as described by Leber (1871) was of a papillitis. Usher (1927b) commented on the presence of haemorrhages at the disc in the acute phase. Atrophy of the optic disc set in after an interval of 2 months or so, but the degree of atrophy gave no indication of the final visual acuity (Batten 1909, Usher 1927b, Lundsgaard 1944). Surgical exploration of the chiasmal area revealed arachnoiditis and a serous meningitis in a number of patients.

Autopsy reports have been published on unquestioned cases. In Rohrbeiner's (1930) account only the eyes and the optic nerves were examined; Kwittken and Benest (1959) claim to have carried out the first complete examination. Further complete pathological examinations have been reported by Wilson (1963), and Adams et al (1966). In all reports there were atrophic changes in the retina

and the optic nerves were extensively demyelinated, the papillo-macular bundles being completely atrophied. Kwittken and Barest (1956) noted that there appeared to be a primary transneuronal degeneration in the whole optic tract except for the calcarine cortex. These authors further described spinal cord symptoms and signs, for which corresponding neuro-pathological changes could be demonstrated. These changes in the spinal cord these authors attributed to malnutrition. Rehsteiner (1930) concluded that the pathological evidence was identical with that found following the toxic atrophy of the optic nerve in diabetes or tobacco amblyopia, and did not resemble the atrophy resulting from retrobulbar neuritis. The majority of the cases described by Wilson (1963) showed manifestations of a diffuse neurological disorder. Long tract signs were present in most and included lower or upper limb paraesthesiae, defective vibration sense, extensor plantar responses, spasticity and hyperreflexia. An eighth nerve dysfunction of varying degree was demonstrable in half the cases. There was no convincing haematological abnormality, but all the cases for whom relevent information was available were smokers.

Additional information of a like nature was presented by Adams et al (1966).

Leber (1871) himself wrote that even unaffected members of a family may suffer from protean neurological disorders such as migraine, giddiness, weakness, or were easily fatigued. This has been amplified by the finding of associated neurological conditions, such as epilepsy, mental retardation, spastic quadripesis, ataxia of cerebellar origin, posterior column loss, various aches and pains, and paranoid schizophrenia. (Taylor 1892, Nettleship 1909, Taylor and Holmes 1913, Ferguson and Critchley 1928, Colenbrander 1962, Walsh 1957, Kwittken and Barest 1958). In most of the reported cases the neurological disorders have developed in the presence of advanced optic atrophy or severe retrobulbar neuritis and usually some time after the initial visual disturbance, however, this was not so in cases described by Bruyn and Went (1964) and Adams et al (1966).

Manifestations of the malady are not confined to the central nervous system - diverse skeletal

abnormalities, a tendency to fracture, arachnodactyly and atypical ankylosing spondylitis have been described (Wilson 1965b).

The pattern of inheritance is puzzling. The reported pedigrees variously show features suggestive of a sex-linked inheritance, autosomal inheritance, or cytoplasmic transmission. Colenbrander (1962) postulated the presence of a "Lebervirus" in the cytoplasm which produced a cellular mutation. Van Senus (1963) felt that an autosomal modifier gene was required for the full expression of a sex linked inheritance. Wilson (1963, 1965a) and Adams et al (1966) feel that the basic condition is an inborn metabolic error, for whose expression exogenous factors are pre-eminent, and that there are relatives of affected persons at risk who are predisposed to the malady but have not encountered the exogenous factor. These authors further postulate cyanide to be the toxic factor.

Wilson (1965a) examined patients suffering from Leber's hereditary optic atrophy for products of cyanide

metabolism, and found significantly reduced concentrations of thiocyanate in the plasma and in the urine, the differences being more marked in those patients who smoked. In this respect the disease resembles tobacco amblyopia. Because of the reported success in treating tobacco amblyopia with hydroxocobalamin (Chisholm, Foulds and Bronte-Stewart, 1967), the following patient suffering from Leber's hereditary optic atrophy was given similar treatment.

This man, aged 35, was seen with a year's history of numbness and paraesthesiae affecting the right side of the body and coincident blurring of vision of the right eye. Similar blurring of vision of the left eye had been present for one month. He smoked 40 cigarettes daily and drank several pints of stout per day. On examination the visual acuity of the right eye was 2/60 (Snellen) and that of the left eye 6/12 (Snellen) with a dense central scotoma in the right field of vision and a smaller centro-caecal scotoma, more striking to a red target, in the left. The right optic disc was pale and the left oedematous. The right plantar response was extensor but there were no other

abnormal neurological signs.

During the next 4 weeks the vision of the left eye deteriorated to 1/60(Snellen) with an increase in the size of the central scotoma. There was striking bilateral optic atrophy. Leber's hereditary optic atrophy was diagnosed when it was discovered that his maternal uncle had been diagnosed as a case of Leber's hereditary optic atrophy in 1937, at the age of 36. The patient was registered as blind. The serum vitamin B12 level was 150 pg. per ml. (Euglena gracilis method) but other laboratory investigations were not significant.

He was treated first with cyanocobalamin 1 mg. intra-muscularly on alternate days for two weeks and then with prednisolone commencing with 40 mg. daily reducing to 5mg. daily over two months, and thereafter at this dosage for eight months longer, but the visual acuity remained unchanged.

Some 2 years later, when the superiority of hydroxocobalamin over cyanocobalamin in the treatment of

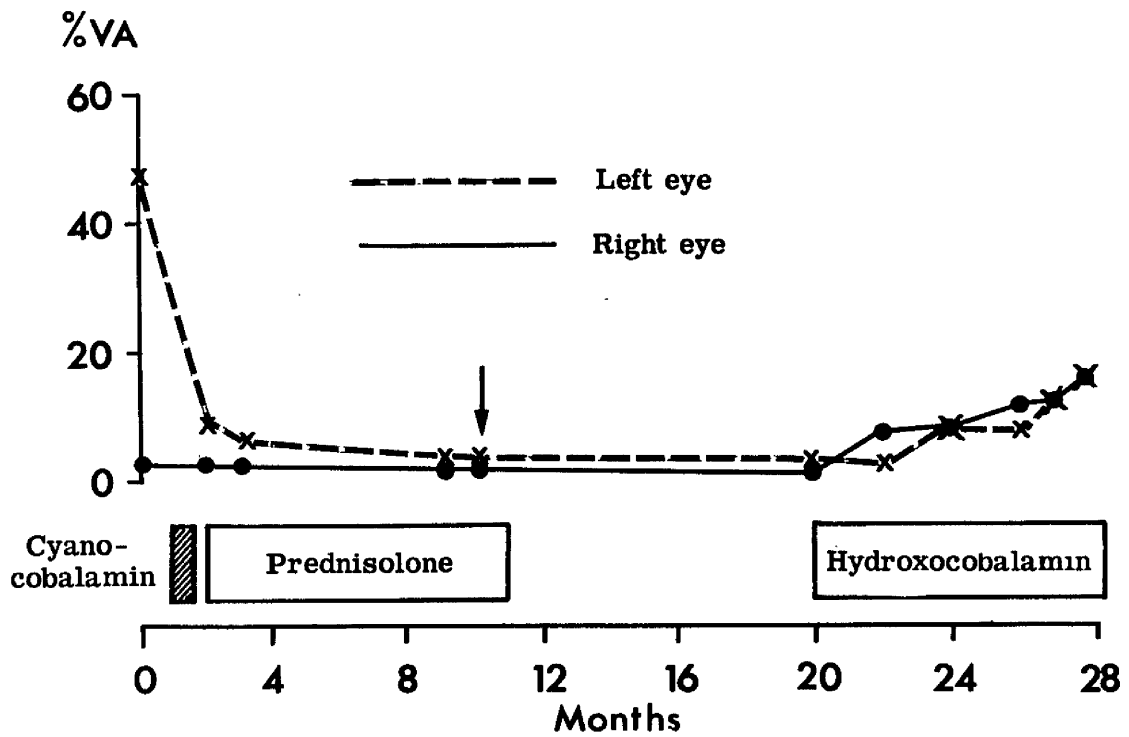


Figure 6, 1. Graphic representation of visual progress in a patient suffering from Leber's hereditary optic atrophy treated with hydroxocobalamin. The arrow indicates the time at which the patient was registered blind.

tobacco amblyopia was realised, the patient was treated with parenteral hydroxocobalamin 1 mg. daily for 2 weeks and thereafter twice weekly. Before starting this treatment the acuity of the right eye was 2/60 and of the left 1/60 (Snellen).

After 3 months the visual acuity had improved to 3/60 in each eye and after 6 months it was 6/60 in the right eye and 6/36 in the left. The patient could now read large print and was no longer considered registrable as blind. The progress of the visual defect is shown graphically in Figure 6, 1. (A published account of this case is located in Appendix B, 2).

Members of the following Spanish family have been examined and treated with hydroxocobalamin. Three of the patients have been followed for five months. All the members examined belong to the third generation (See Fig.6,2).

III, I. A male aged 34 years was examined in October, 1968. There was a history of sudden bilateral loss of vision whilst on a business trip 6 years previously. The

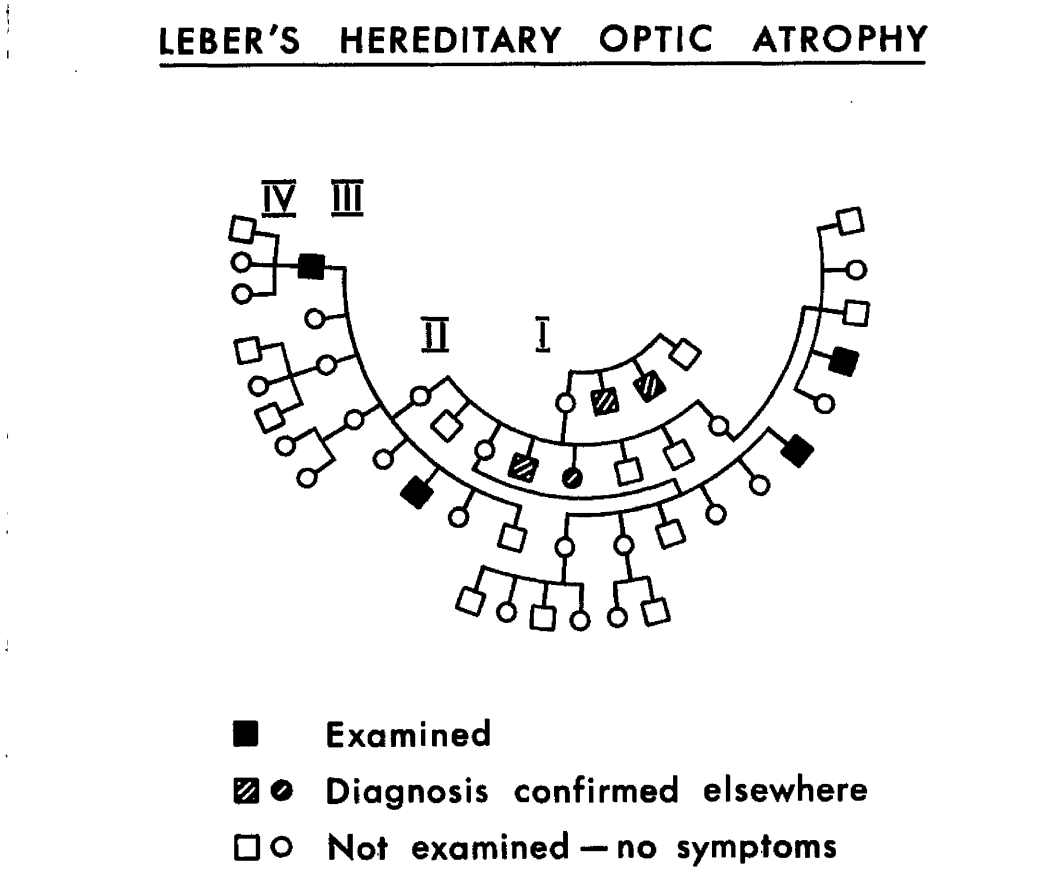


Figure 6, 2. Pedigree of Leber's Hereditary Optic Atrophy.

visual loss had been preceeded by an influenza-like illness of one-two days duration. He smoked 20 or more cigarettes per day. The year following the visual loss he had undergone craniotomy for chiasmal arachnoiditis. The visual loss was severe and had remained so.

Examination revealed a corrected visual acuity in the right and left eyes of 3/60 (Snellen); with a x4 low visual aid his near vision was N5. Ophthalmoscopic examination revealed marked pallor of the optic discs, with sheathing of the retinal vessels at the disc in each eye. The ocular media were clear and the maculae normal. Examination of the central field of vision revealed a central scotomata, extending to 10^{0} around fixation in the right eye and 3^{0} in the left eye. (Figure 6,3). The scotomata were confirmed by static perimetry. The Farnsworth-Munsell 100 Hue test result gave an error of 560 for right eye, 520 for left eye.

General examination revealed no systemic or neurological abnormality. Electro-encephalographic examination revealed a normal tracing. The haemoglobin

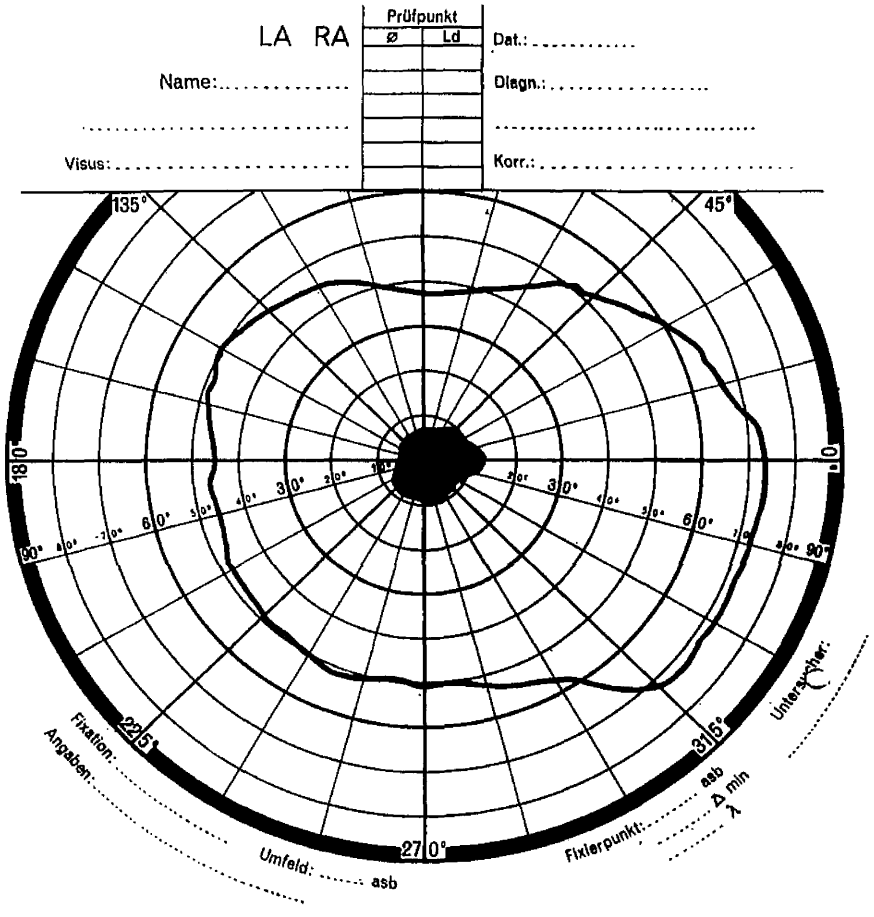


Figure 6, 3. Central area of the visual field in untreated Leber's hereditary optic atrophy (Patient III, I).

concentration was 116%; serum vitamin B12 306 pg/ml; serum folate 4.4ng/ml; a weak +ve result on testing for gastric parietal cell antibody; and a -ve result on testing for antibody to intrinsic factor; the Schilling test showed 33% recovery.

Treatment with hydroxocobalamin was commenced with 5 mgm. daily for 14 days and then reduced to 1 mgm. thrice weekly thereafter. After 5 months on this treatment there was subjective improvement in visual acuity, not confirmed on the Snellen chart, but confirmed by static perimetry which showed a reduction in the size of the scotoma.

III, 6. A male aged 24 years was examined in October, 1968. There was a history of sudden bilateral loss of vision 4 years previously while studying for examinations. There was no precipitating illness. He smoked upwards of 20 cigarettes per day.

Examination revealed a corrected visual acuity right eye 2/60, left eye 1/60 (Snellen) with x4 low visual

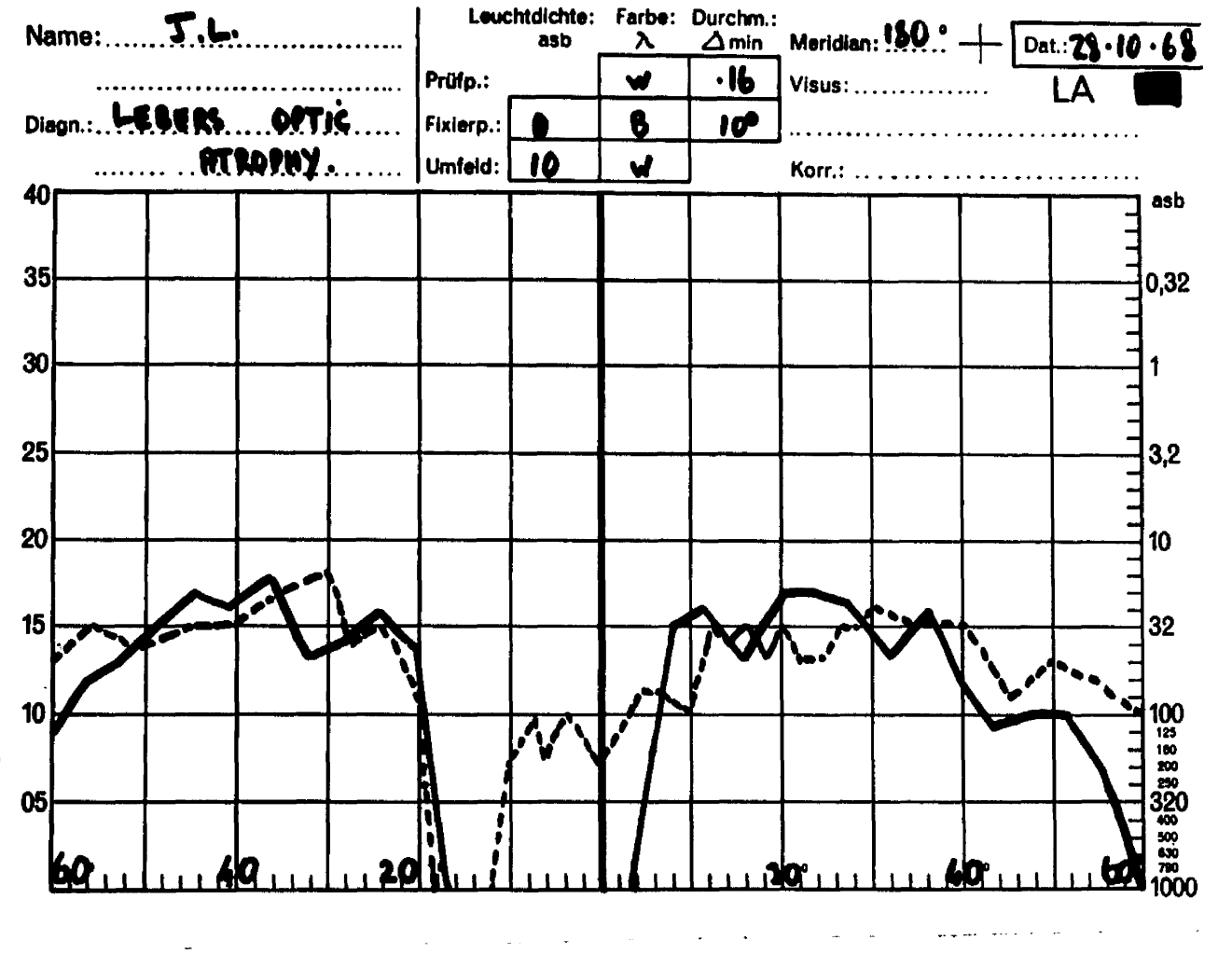


Figure 6, 4. Showing the alteration in the static perimetry chart of the left eye before (-) and after 5 months therapy with hydroxocobalamin (---). There is shrinkage in the area of the scotoma. (Patient III, 6).

aid he was able to read N5 with either eye. Ophthalmoscopy revealed bilateral pallor and atrophy of the optic discs with sheathing of the retinal vessels at the disc. The ocular media were clear and the maculae normal. Examination of the central field of vision revealed a central scotoma extending to 3° from fixation in the right eye and 10° in the left eye. The scotomata were confirmed by static perimetry. The Farnsworth Munsell Hundred Hue test result gave an error of 244 for right eye and 529 for left eye (Fig. 6,5).

General examination revealed no systemic neurological abnormality. Electro-encephalographic examination revealed a normal tracing. The haemoglobin concentration was 113%, serum vitamin B12 446 pg/ml, serum folate 3.7 ug/ml, a negative result on testing for antibody to gastric parietal cells and intrinsic factor, the Schilling test result showed 18% recovery.

Treatment with hydroxocobalamin was commenced with 5 mgm. daily for 14 days and followed with 1 mgm. three weekly. After 5 months on this treatment there was a

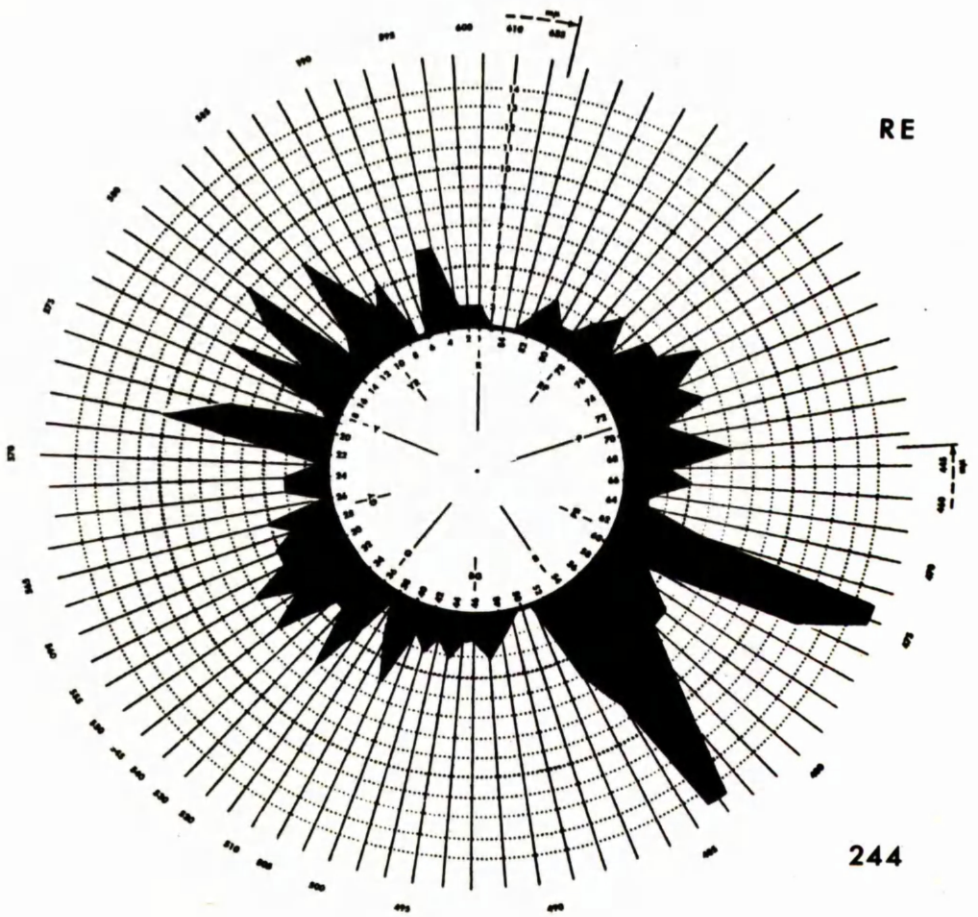


Figure 6,5. Typical Farnsworth Munsell 100 Hue chart in Leber's Hereditary Optic Atrophy.

subjective improvement. The corrected visual acuity had improved in the right eye to 3/60 and left eye to 2/60 (Snellen). There has been improvement on static perimetry examination (Figure 6,4).

III. 14. A male aged 16 years was examined in March, 1969. There was a history extending over the previous 6 months. The visual acuity of the right eye failed first without any discoverable ophthalmic cause, the vision in the left eye deteriorated some 3 weeks before examination. An ophthalmologist at the patient's home town had commenced therapy with systemic steroid (Prednisolone) and a multi vitamin preparation - which included cyanocobalamin. The patient smoked 10 cigarettes per day.

The corrected visual acuity of the right eye was 1/60 and that of the left eye 6/18, (Partly) (Snellen). Ophthalmoscopy revealed hyperaemia of both optic discs with vascular engagement of the upper halves of each disc. No vascular sheathing was observed in either eye. Fluorescence angiography confirmed the presence of small vessel dilatation of the upper halves of each optic disc, from which no leakage of dye occurred during the dye transit.

The examination of the central fields showed a characteristic central scotoma in the right eye and a centro-caecal scotoma in the left eye, which were confirmed by static perimetry. The Farnsworth Munsell 100 Hue test gave an error score of 556 with the right eye and 316 with the left eye.

General examination revealed no systemic or neurological abnormality. Electro-encephalographic examination gave a normal tracing. The haemoglobin concentration was 122%, serum vitamin concentration was normal, serum folate 5.9 ug/ml, a negative result in testing for gastric parietal cell antibody, the Schilling test result showed 6.9% recovery.

The patient was treated with the following intensive regime:-

Day 1. 10 mgm. hydroxocobalamin given as an intravenous infusion in 500 ml. saline.

Day 2. 5 mgm. hydroxocobalamin given by intramuscular injection.

Day 3. Repeat of the intravenous infusion with 10mgm. hydroxocobalamin.

The patient's previous therapy with systemic steroid was continued throughout at the same dose. By day 4 the visual acuity of the left eye had risen to 6/9 (partly) Snellen, and the therapy was continued with hydroxocobalamin 5 mgm. thrice weekly but in spite of this the visual acuity fell to 6/36 (Snellen) over the next week. The dose of hydroxocobalamin was increased to 5 mgm. daily without any additional improvement in vision. Although the visual acuity fell after the initial improvement, the observed improvement in the visual field has been maintained.

This patient has been studied in the acute early phase of Leber's hereditary optic atrophy. His present therapy consists of hydroxocobalamin 5 mgm. daily, Prednisolone 5 mgm. thrice daily. He has been advised to stop smoking.

III. 16. A male aged 14 years was examined in October, 1968. There was a history of abrupt loss of vision in both eyes when aged 10 years. He had undergone surgery for a mastoiditis at 7 years of age. He was a non-smoker. Professor Frauschettti had prescribed male hormone injections with no benefit.

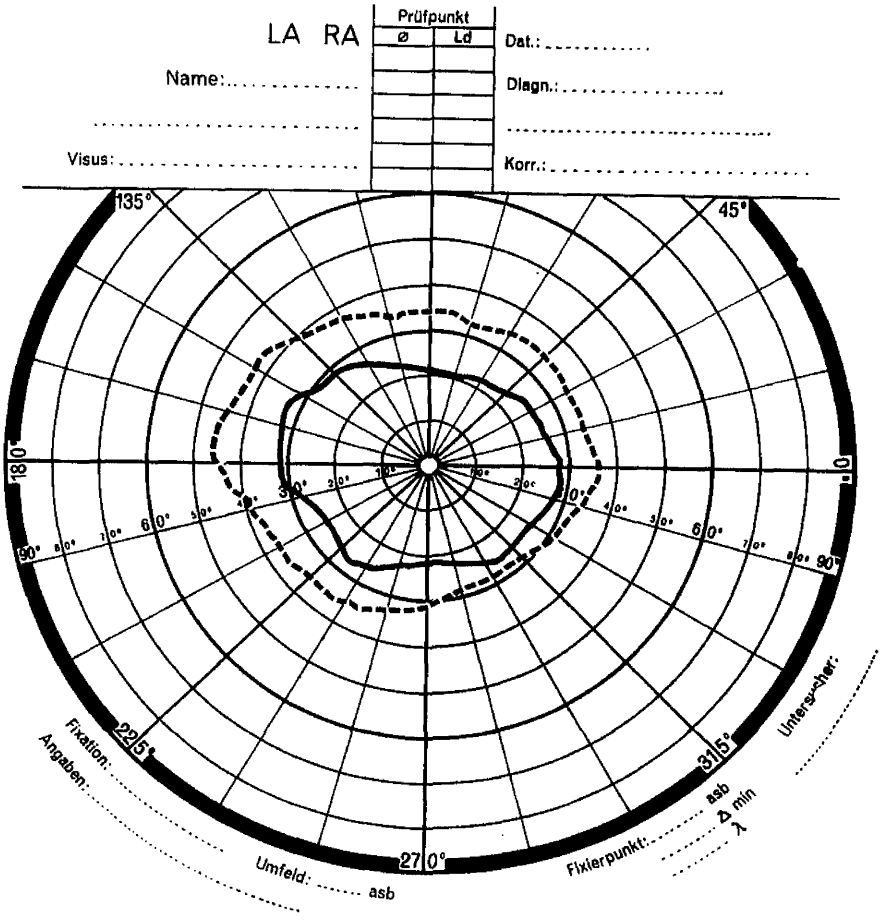


Figure 6, 6. Leber's hereditary optic atrophy showing the peripheral visual field before (-) and after (---) hydroxocobalamin therapy. (Patient III, 16, left eye).

Examination revealed a corrected visual acuity in the right eye and left eye of 1/60 (Snellen) and with his low visual aid he managed N6. Ophthalmoscopic examination revealed marked pallor of the optic disc and sheathing of the retinal vessels on the disc in each eye. Examination of the visual fields revealed peripheral constriction to both colour and white targets and a dense absolute scotoma extending to 3° about the fixation point. The Farnsworth Munsell 100 Hue test gave an error of 1195 for the right eye and 1320 for the left eye.

General examination revealed no systemic or neurological abnormality. Electro encephalographic examination revealed a normal tracing. The haemoglobin concentration was 113%, serum vitamin B12 500 pg/ml, serum folate 5.5 ng/ml., a negative result on testing for gastric parietal cell antibody and antibody to intrinsic factor; the Schilling test result showed 7.9% recovery.

Treatment with hydroxocobalamin was commenced with a dose of 5 mgm. daily for 14 days and then continued

with 1 mgm. thrice weekly thereafter. At the end of 5 months there was subjective visual improvement in visual acuity which was not confirmed on the Snellen chart, but was on static perimetry and there was less constriction of the periphery of his visual field (Fig. 6,6).

Examination of these patients for defects in vitamin B12/cyanide relationships was carried out. The relative data are contained in Table 6, 1 and 6, 2. As previously reported (Foulds et al 1968c) no significant abnormalities were detected in serum vitamin B12 assay, or Schilling test results in Leber's hereditary optic atrophy. The mean plasma cyanide concentration was 0.034 micro moles. When only the smokers were considered the mean cyanide concentration was 0.045 micro moles. A similar higher level was found in the smokers, when the plasma thiocyanate concentrations were examined - mean 2.88 micro moles per 100 mls, 3.18 micro moles per 100 mls. for smokers only, and in the urine where the thiocyanate concentrations were 3.27 micro moles/100 mls. urine and, 3.75 micro moles/100 mls. urine for the smokers.

Table 6,1.

LEBER'S HEREDITARY OPTIC ATROPHY - UNTREATED

	Age	Tobacco consumption	Plasma Cyanide	Plasma Thiocyanate	24 hour Urinary Thiocyanate	Thiocyanate Clearance	Serum Folate
111, 1	35	20 cigs/day	0.056	3.3	30.87	0.65	4.4
111, 6	24	30-40 cigs/day	0.048	4.4	44.2	0.82	3.7
111, 14	16	10 cigs/day	0.03	1.85			5.9
111, 16	14	N.S.	0.001	2.0	23.0	0.79	5.5

Table 6,2.

URINARY EXCRETION OF THIOCYANATE/100 mls, AND RENAL CLEARANCE OF THIOCYANATE WITH TIME

	0	6 days	10 days	14 days	6 weeks	5 months
111, 1	3.25/0.65		9.2/1.33			9.86/42.02
111, 6	4.25/0.82		5.5/1.24			10.0/6.2
111, 14		1.8/1.6		3.3/3.05	13.0/5.7	
111, 16	2.30/0.79		0.6/1.25			1.0/2.5
Mean Urinary Thiocyanate	3.27		5.1			6.95
Renal Thiocyanate Clearance	0.75		1.27			16.91

As demonstrated in the patients suffering from tobacco amblyopia there is a rise in the urinary excretion; and a rise in the renal clearance for thiocyanate, on treatment with hydroxocobalamin. The mean urinary thiocyanate was found to be 3.27 micro moles per 100 mls. of urine and the clearance for thiocyanate to be 0.75 mls. per minute prior to treatment. On giving hydroxocobalamin by injection at a dose of 5 mgm. daily for 2 weeks followed by 1 mgm. thrice weekly, the mean urinary excretion rose to 5.1 micro moles per 100 mls. of urine after 10 days, and to 6.95 micro moles per 100 mls. of urine after 5 months therapy. There was a similar rise in the renal clearance for thiocyanate from 0.75 mls. per minute to 1.27 mls. per minute after 10 days, and 16.91 mls. per minute after 5 months. The rise in both the urinary concentration of thiocyanate and the renal clearance of thiocyanate was more marked in those patients who smoked. From figure 6,7, it can be observed that the urinary excretion of thiocyanate rises with time on treatment as does the renal clearance of thiocyanate. As far as the patients have been followed up neither factor has reached its peak. These results contrast with results for urinary

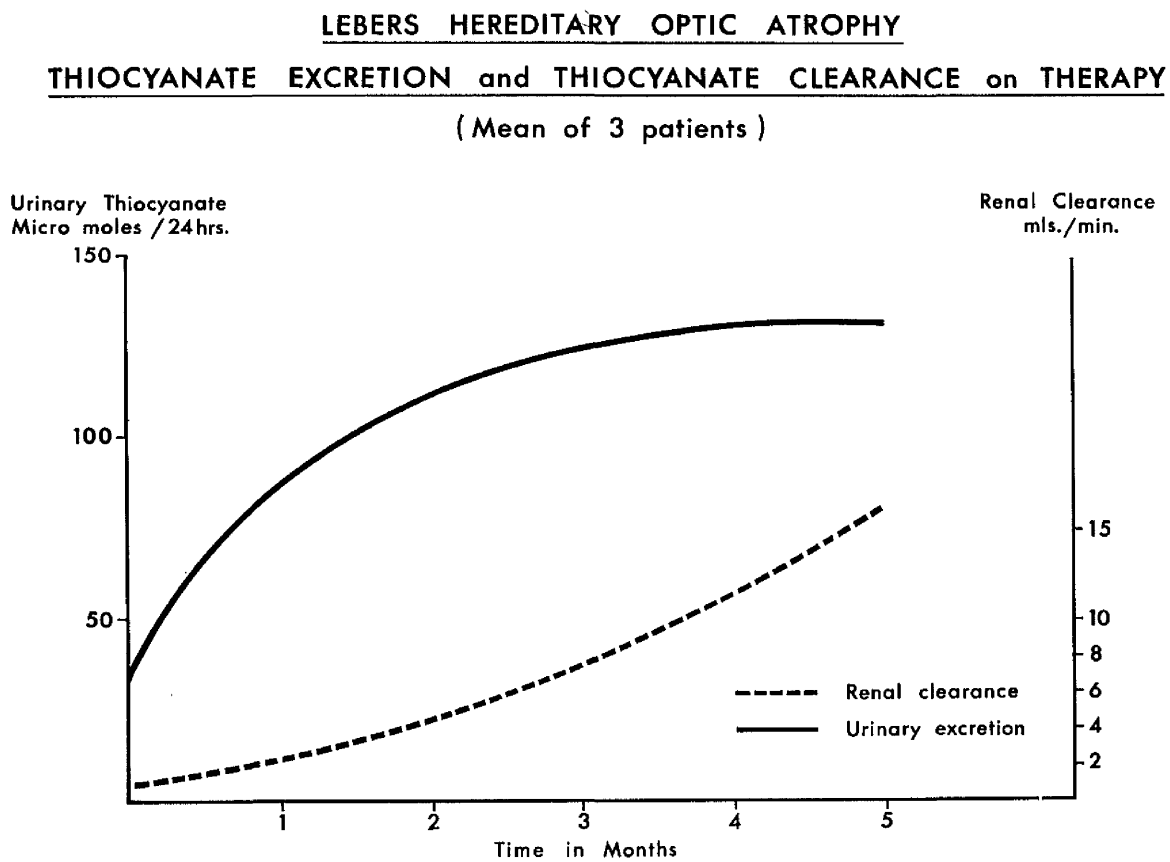


Figure 6, 7. Alteration in urinary excretion of thiocyanate (—) and of renal clearance of thiocyanate (---) in Leber's hereditary optic atrophy.

excretion obtained in the tobacco amblyopia patients whose thiocyanate excretion reached a peak some two weeks after the commencement of therapy.

II. Association between Tobacco Amblyopia and Addisonian Pernicious Anaemia.

The association between these two conditions has been recognised for some time. Thus Leishman (1952) found 10 examples of addisonian pernicious anaemia in his review of 75 patients suffering from tobacco amblyopia, and Heaton et al (1958) 2 examples in their analysis of 13 tobacco amblyopia patients. The latter authors further felt that patients with the retrobulbar neuritis of pernicious anaemia who smoked could equally be described as suffering from tobacco amblyopia. This theme was further amplified and confirmed by Freeman and Heaton (1961). Leishman (1951) was of the opinion that tobacco amblyopia and the retrobulbar neuritis of pernicious anaemia could co-exist and Cohen (1936) and McAlpine and Goldsmith (1951) found optic atrophy as the presenting sign of pernicious anaemia.

Callender and Denborough (1957) produced evidence that there is a "pre-pernicious anaemia" state in which there is laboratory evidence of pernicious anaemia (i.e. achlorhydria, poor absorption of vitamin B12, low or low normal serum vitamin B12 concentration) without anaemia, and which precedes the anaemia by an interval of years (Hurst 1922). Patients fitting this pre-pernicious anaemia state were found both by Leishman (1951) and Heaton et al (1958) in their studies.

Turner (1940) noted that the development of amblyopia in pernicious anaemia was not related to the degree of anaemia, but was possibly related to the smoking habit of the patient. Adams et al (1967) and Foulds et al (1968a) reported examples of amblyopia in non-smokers. Of the 3 patients reported by Foulds et al (1968a) all were female.

In this present analysis of 65 patients suffering from tobacco amblyopia 11 patients were found to suffer from Addisonian pernicious anaemia, diagnosis being based on anaemia (haemoglobin less than 100gm%), megaloblastic

erythropoesis in marrow examination serum vitamin B12 concentration 110 pg/ml, circulating antibody to gastric parietal cells, achlorhydria, and a Schilling test result of 6 recovery. In a further 11 patients evidence of the "pre-pernicious anaemia" state without anaemia was found. Of these patients 1 subsequently proved to have a gastric carcinoma and 4 had undergone previous gastric or intestinal surgery. Thus slightly more than $\frac{1}{3}$ of the patients in this analysis showed evidence of overt or occult pernicious anaemia.

The patients suffering from Addisonian pernicious anaemia were Nos. 1,7,8,13,17,27,29,33,36,50 and 57, and included the only female patient in the analysis who smoked cigarettes (those in the "pre-pernicious anaemia" state were Nos. 6,18,20,23,25,44,46,48,56,58,63.

None showed the retinopathy of pernicious anaemia and ophthalmoscopy revealed pallor of the optic discs. Examination of the visual fields revealed the centro-caecal scotoma of tobacco amblyopia and the examination of the colour sense showed a gross disturbance. Several patients

had been diagnosed as suffering from pernicious anaemia initially and had the visual defect categorised subsequently.

The clinical details of three of the cases merit mention individually. These are two cases of tobacco amblyopia occurring in patients shown to have pernicious anaemia and whose vision failed to improve on treatment with cyanocobalamin in spite of a return to normality of the blood picture. In each case there was a prompt improvement in vision when treatment was changed to hydroxocobalamin. The third patient actually developed tobacco amblyopia while on treatment with cyanocobalamin for pernicious anaemia and who only recovered vision when the treatment was changed to hydroxocobalamin.

The first patient (Case No.1) was a man of 56 years of age who smoked 1 oz. of tobacco per week as cigarettes. He was a vegetarian. When first seen he had a visual acuity of 5/60 with the right eye and 3/60 with the left eye. Examination of the visual fields revealed the characteristic changes of tobacco amblyopia. He was not anaemia (haemoglobin 14 g/100 ml, PCV 45) but the serum B12

level was less than 25 ugs/ml, the folate being normal (11 mug/ml). Examination of the sternal marrow revealed megaloblastic erythropoesis. There was a histamine fast achlorhydria, an abnormal Schilling test (5.2%) and serology showed the presence of antibodies to gastric parietal cells. Examination of the fundi showed some degree of optic atrophy and the patient admitted that his vision had been poor for at least nine months. Treatment with cyanocobalamin 1000 ugs. daily for one week was started and this was followed by 500 ug. fortnightly for six months. Normoblastic erythropoesis was restored but the visual acuity remained unaltered and the patient's treatment was therefore changed to hydroxocobalamin 1000 ug. fortnightly. Again there was a rapid improvement in vision, particularly of the right eye, which within a month had improved to 6/18 and has continued to improve reaching 6/6 in six months. The vision in the left eye has been slower to respond, improving from 2/60 to 6/12 over the course of six months. The progress of the visual defect in each of these cases is recorded in figure 6.8.

The second patient (Case No.7) a male aged 71 years, smoked 2 oz. of pipe tobacco per week. His visual acuity when first seen was less than 6/60 with either eye and there

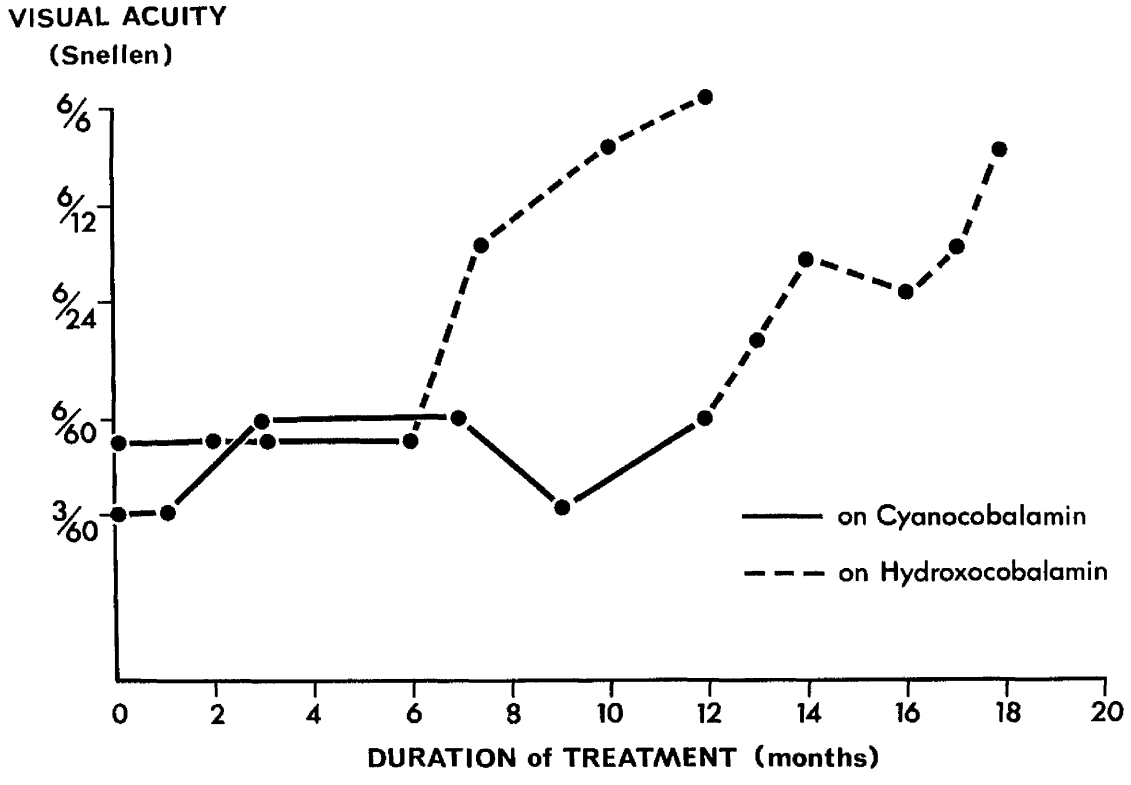


Figure 6, 8. Graphic representation of visual improvement of right eye in patient No.1 and patient No.7, on changing therapy from cyanocobalamin to hydroxocobalamin.

was a field defect characteristic of tobacco amblyopia. The patient was anaemic (haemoglobin 7.0 g/100 ml., PCV 24.5) and was subsequently shown to be suffering from Addisonian anaemia (achlorhydria, megaloblastic marrow, positive serology for gastric parietal cell antibodies, schilling test 3.7%). Treatment with parenteral cyanocobalamin 1000 ugs. once weekly for one month was instituted and was followed by 1000 ugs. once per fortnight for seven months when the dose was reduced to 500 ugs. at intervals of three weeks. On this treatment the blood picture returned rapidly to normal, but the visual acuity which initially improved over a period of two months, from less than 6/60 to 6/60 in each eye, did not improve further. After one year on cyanocobalamin treatment was changed to hydroxocobalamin (1000 ugs. twice per week). There was an almost immediate improvement in visual acuity. Within two weeks the vision had improved to 6/36 with the right eye and 6/24 partly with the left, and the vision has continued to improve since then reaching 6/9+ with the right eye and 6/12 partly with the left eye in six months.

The third patient (Case No.36) (J.H.) a male

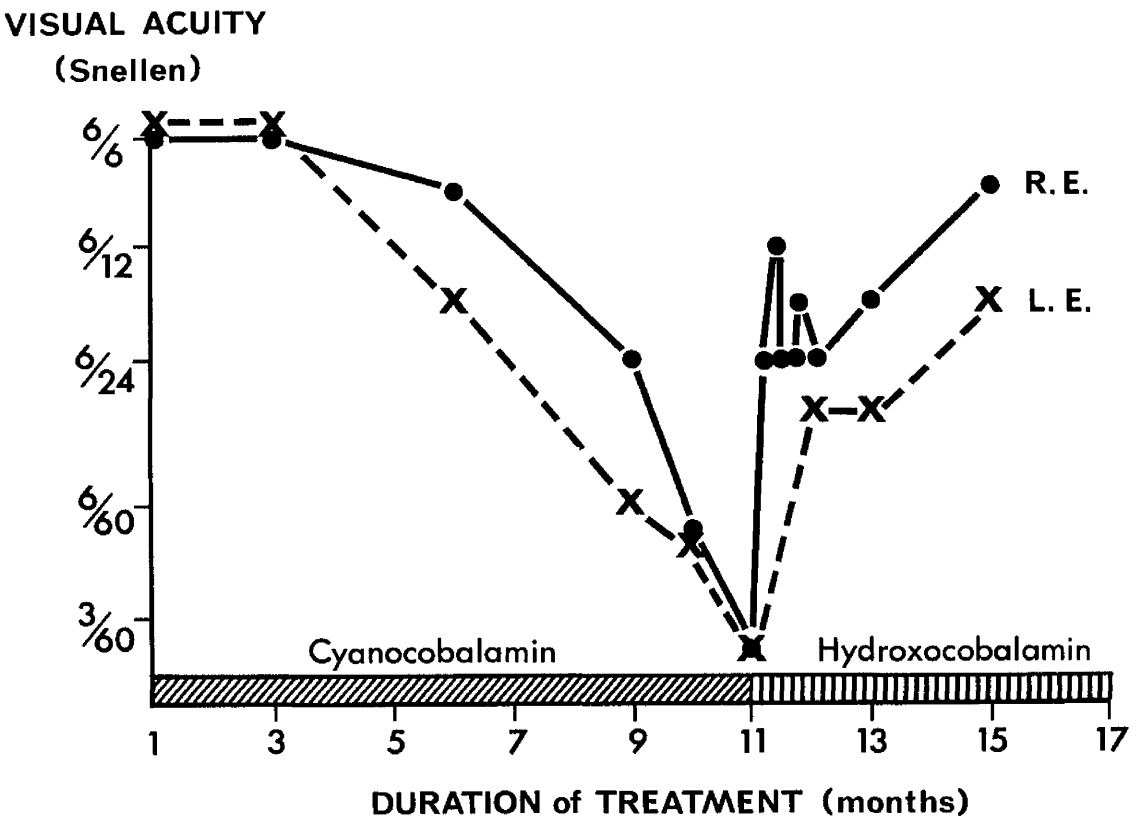


Figure 6.9. Graphic representation of visual acuity of right and left eye whilst on treatment with cyanocobalamin and later hydroxocobalamin. Patient No.36.

pipe smoker, of 51 years of age, smoked one half to three quarters of an ounce of tobacco per week. In May, 1966, he sustained a contusion injury to the back of his head and following this noted a proptosis of the left eye without ecchymosis. He was admitted to hospital where he was found to have pernicious anaemia and treatment with cyanocobalamin was started (1000 ug. daily for one week followed by 250 ugs. weekly for one month, when the dose was reduced to 250 ug. fortnightly. Four weeks later the dose was further reduced to 250 ug. monthly). On this treatment the blood picture returned rapidly to normal. His proptosis however increased and he developed diplopia. In July 1966 he was admitted to the Glasgow Institute of Neurological Sciences where he was found to have 6 mm. of left proptosis, slight blurring of the optic disc in the left eye and a bilateral depression of the centro-caecal area of the visual fields to a small red target, suggestive of early tobacco amblyopia. At that time the visual acuity was normal in each eye. In August 1966 an exploration of the left orbit was carried out, a biopsy showing a non-specific orbital granuloma. Following this procedure the proptosis disappeared and has not recurred. In October 1966

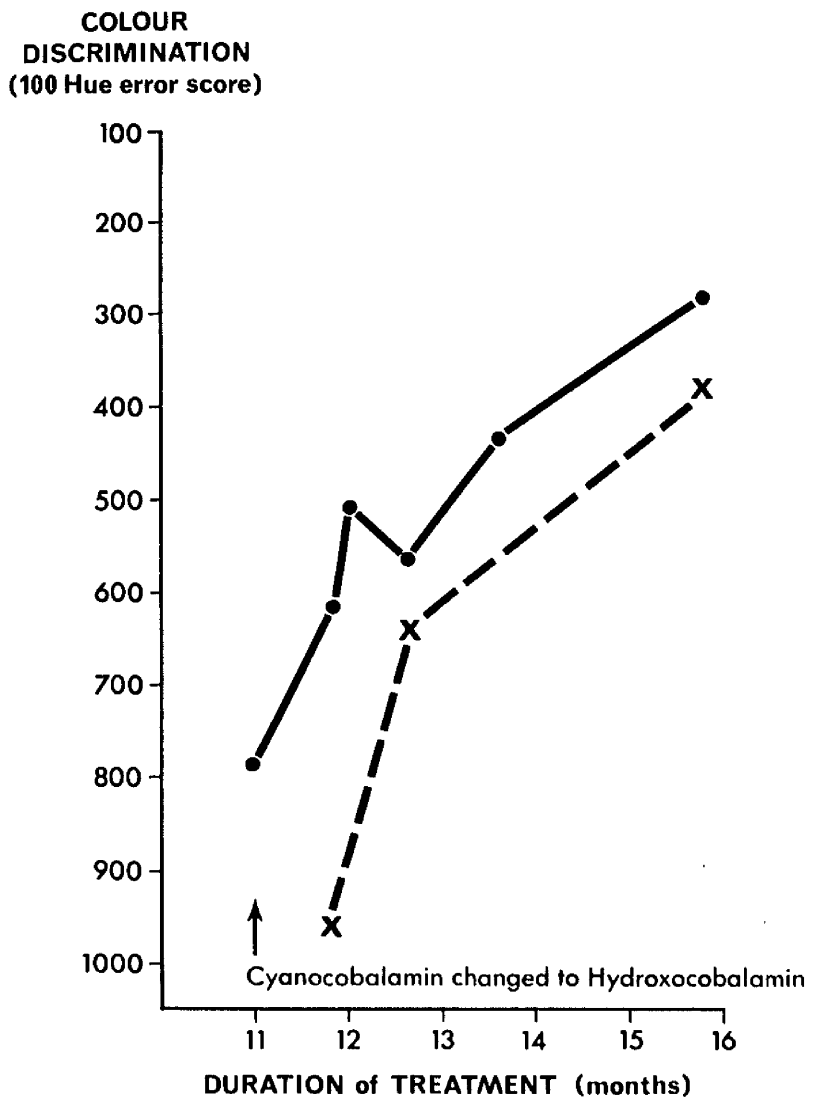


Figure 6.10. Improvement in 100 Hue error score whilst on treatment with hydroxocobalamin. Right ——. Left eye x------x. Patient No. 36.

the visual acuity was noted as 6/9 partly with the right eye and 6/18 with the left. Four months later he was seen again and the visual acuity in the right eye was recorded as 6/24 partly while the vision in the left eye had fallen to 6/60. His reading vision was grossly impaired and the visual fields showed gross centro-caecal loss with dense islands of scotoma typical of tobacco amblyopia. There was no peripheral loss of visual field; the right optic disc appeared normal but the left was slightly pale. The patient was transferred to our care for further investigation when it was found that the visual acuity had fallen to 2/60 (corrected) with either eye, the reading acuity in the right eye being N24 and in the left N48. There was a gross defect of colour discrimination, the Farnsworth Munsell 100 Hue score for the right eye being 796, a partial tarsorrhaphy on the left side making colour vision testing in this eye difficult. During all the time that the patients vision had been deteriorating, treatment with parenteral cyanocobalamin had been maintained and the patient continued to smoke.

Treatment with hydroxocobalamin 1000 ug. daily was started and within three days the visual acuity in the right

eye which had been 2/60 improved to 6/24 partly. One week after the commencement of treatment the visual acuity in the right eye had improved to 6/12, subsequently fluctuating during the succeeding week between 6/18 and 6/24. Two weeks after the commencement of therapy the left tarsorrhaphy was divided. The course of the visual acuity for each eye is shown in Fig.6,9 where it will be seen that over a period of four months the visual acuity in the right eye improved to 6/9 and in the left to 6/18 partly. There was a coincident improvement in colour discrimination which is shown graphically in Fig.6,10. These patients illustrate the ineffective value of cyanocobalamin in the treatment of pernicious anaemia accompanied by visual disturbance. They further support the view that hydroxocobalamin should replace cyanocobalamin in the treatment of pernicious anaemia, particularly if the patient is a smoker.

CHAPTER VII.

CONCLUSIONS

The hypothesis that inter connected disturbances of cyanide/vitamin B12 metabolism may be concerned in the pathogenesis of tobacco amblyopia, the retrobulbar neuritis of pernicious anaemia, Leber's hereditary optic atrophy and certain tropical neurological syndromes apparently associated with a high cyanide intake, is supported by the investigations of Smith (1961) Wilson (1965a), Montgomery (1965) Wilson and Langman (1966), Monekosso and Wilson (1966), Freeman (1967), Chisholm et al (1967) and Osuntokun et al (1969).

Observations apparently providing a direct link between smoking and vitamin B12 metabolism are few. They include:-

1. There may be a slight or moderate increase in cyanocobalamin in the plasma of some heavy smokers. (Lindstrand et al 1966).

2. The finding that a larger proportion of serum

vitamin B12 is extractable in the absence of added cyanide in smokers than in non-smokers. (Smith 1961).

3. The finding of a negative correlation between plasma cyanide and total serum B12 concentrations. (Matthews et al 1965, Wilson and Matthews 1966).

4. The urinary excretion of vitamin B12 is increased in smokers, and is associated with an increase in vitamin B12 excretion and a relatively low serum vitamin B12 concentration (Wilson and Matthews 1966).

The findings contained in this thesis are presented to amplify and strengthen the theory underlying the aetiology of tobacco amblyopia and related conditions - namely a disturbance in the cyanide / vitamin B12 relationship and the following new facts may be emphasised.

(a) The concentrations of serum vitamin B12 in tobacco amblyopia are reduced when compared with the concentrations in non-amblyopic smokers and non-smokers.

(b) There is a positive correlation between tobacco intake, on the one hand and serum vitamin B12 concentrations, and vitamin B12 absorption on the other, in subjects suffering from tobacco amblyopia.

(c) There are reduced concentrations of thiocyanate in the blood of tobacco amblyopes which undergo elevation in response to therapy with hydroxocobalamin.

(d) A rise in the renal excretion of thiocyanate in tobacco amblyopia occurs in response to therapy with hydroxocobalamin.

(e) The elevation of the thiocyanate concentration in the blood and urine is also found in patients suffering from Leber's hereditary optic atrophy who are treated by hydroxocobalamin.

(f) Changes in the renal clearance of thiocyanate observed in tobacco amblyopia, Leber's hereditary optic atrophy and non-amblyopic smoking subjects, receiving hydroxocobalamin can only be explained by the renal tubular epithelium being a site of production of thiocyanate.

If the intestinal absorption of vitamin B12 is reduced to such a degree that the daily metabolic turnover rate or loss of vitamin B12 (approximately 2.55 ug per day, Heinrich 1968) is no longer compensated for, only the vitamin B12 pools in the storage organs and tissues can be

used as a source for the metabolic vitamin B12 requirement. This situation results in a progressive depletion of the vitamin B12 coenzyme body stores, which is reflected in a reduction of the vitamin B12 concentrations in the serum and urine and an increased methyl malonate urinary excretion.

Following manifestation of the biochemical vitamin B12 deficiency symptoms, the morphological and clinical symptoms of B12 avitaminosis can become manifest (Fig.7.1)

There is ample evidence by other authors and in this thesis that, in general, tobacco amblyopia patients have a lower serum vitamin B12 concentration than do non-amblyopic smokers, though in only 16% was the serum concentration found to be pathologically low. A significant proportion of the tobacco amblyopes showed, in addition a defective absorption of radioactive labelled vitamin B12.

It has been shown that cases of tobacco amblyopia, whether overtly vitamin B12 deficient or not, recover vision on treatment with hydroxocobalamin, even if smoking is continued (Chisholm et al 1967). From this alone it is

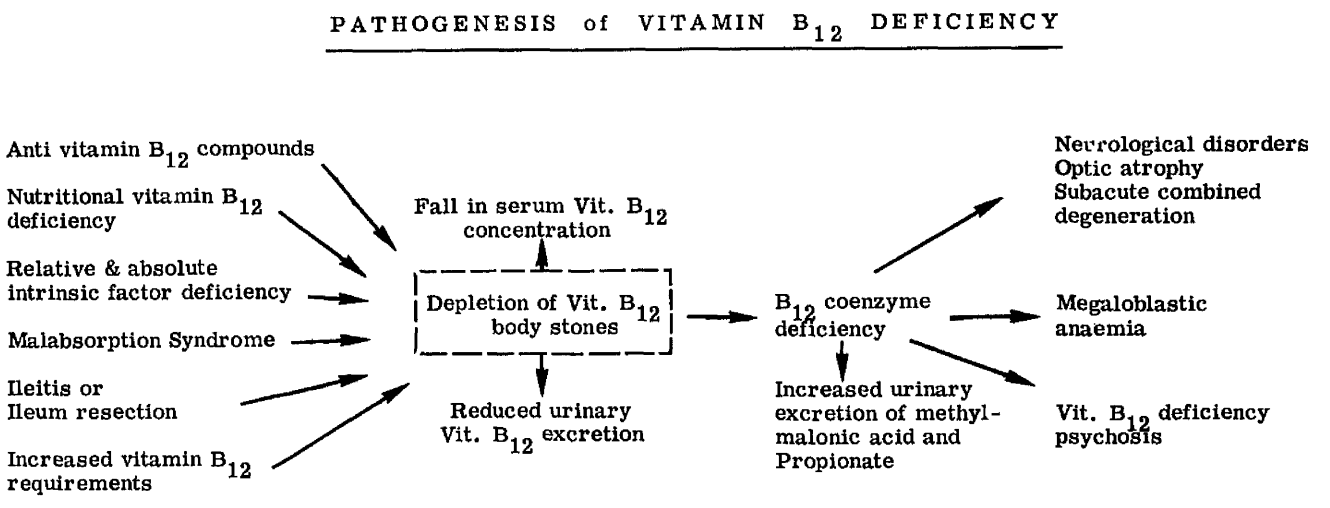


Figure 7.1. Summary of vitamin B12 deficiency.

evident that in these patients, although there may be no apparent vitamin B12 deficiency, there must be a relative deficiency of vitamin B12 itself or some active vitamin B12 analogue.

The development of tobacco amblyopia, however, cannot be merely the consequence of a high cyanide intake in the presence of vitamin B12 depletion, for the correlation between tobacco consumption and serum vitamin B12 concentration is not very close. In addition, examination of a number of patients with Addisonian pernicious anaemia who were also heavy smokers, has shown no discoverable disturbance of visual function. If vitamin B12 deficiency and heavy tobacco consumption were the sole factors in the production of the amblyopia, such patients would inevitably develop the condition.

The results do not reflect the view that tobacco consumption may depress vitamin B12 absorption. There is no statistical difference in the serum vitamin B12 concentration of smoking and non-smoking subjects and there is no correlation between tobacco intake and serum vitamin

B12 concentration in healthy pipe smokers. (Only in the amblyopic patient do the intestinal absorption and serum concentrations of vitamin B12 vary directly with tobacco intake). It would seem from the results that depressed absorption of vitamin B12 as shown by the Schilling test or low serum concentrations of vitamin B12, is an independently determined factor of aetiological significance in relation to the development of tobacco amblyopia.

It is well recognised that patients suffering from tobacco amblyopia show a high incidence of systemic disease, especially of those diseases known to be associated with abnormalities of vitamin B12 metabolism. Even in patients with a normal capacity for absorbing vitamin B12, the serum vitamin B12 is often low, and the results suggest that there is often a dietary insufficiency, which is further reflected by the finding of low serum concentrations of other factors such as folic acid.

The therapeutic effect of hydroxocobalamin in these diseases is difficult to explain and requires examination of the known detoxication mechanisms for cyanide.

1. Cyanide is combined with inorganic sulphur in the liver to form thiocyanate. This reaction is catelysed by the enzyme thiocyanate-cyanide sulphur transferase. (Rhodanese, EC. 2.8.1.1.)

2. Cyanide may react directly with 3 mercapto - pyruvate to yield thiocyanate and pyruvate. This reaction is catalysed by the enzyme 3 mercapto pyruvate - cyanide sulphur transferase. The 3 mercapto pyruvate is obtained by transamination of l-cysteine.

3. Cyanide is known to react spontaneously with cystine to yield cysteine and either 2 - imino 4 - thiazolidine carboxylic acid, or B-thiocyanoalanine. Little is known of the further reactions of 2-imino, 4 thiazolidine carboxylic acid, but B - thiocyanoalanine after deamination will yield thiocyanate and pyruvate.

4. Incorporation into the carbon - one metabolic pool and subsequent excretion as carbon dioxide or synthesis into formate, allantoin, choline etc.

5. Direct union with hydroxocobalamin to yield cyanocobalamin.

The therapeutic effect of hydroxocobalamin has been shown to bring about a rise in the thiocyanate

concentration in the blood and urine. Somewhat similar changes have been shown to occur also in patients suffering from the optic neuropathy of pernicious anaemia, and Leber's hereditary optic atrophy on the same treatment. It would be naive to assume that the therapeutic effect of hydroxocobalamin is merely its union to form cyanocobalamin, as Smith (1968) has shown that the amount of cyanide removable by such means is very small. It would seem unlikely also, that hydroxocobalamin was acting as a "carrier" for cyanide, as the cyanide cobald bond is stable, and cyanocobalamin is metabolically inactive and is excreted unchanged in the urine. (Smith 1968). A more rational consideration would be that therapeutic hydroxocobalamin replenishes the depleted adenosyl coenzyme vitamin B12 stores necessary for the preparation of a suitable sulphur donor for the conversion to thiocyanate of cyanide.

Dietary sulphur is ingested as protein containing the sulphur amino acids, cystine, cysteine and methionine. Such sulphur containing amino acids are utilised in the synthesis of tissue protein such as hair, insulin, glutathione, B mercapto-ethanolamine and taurine and some

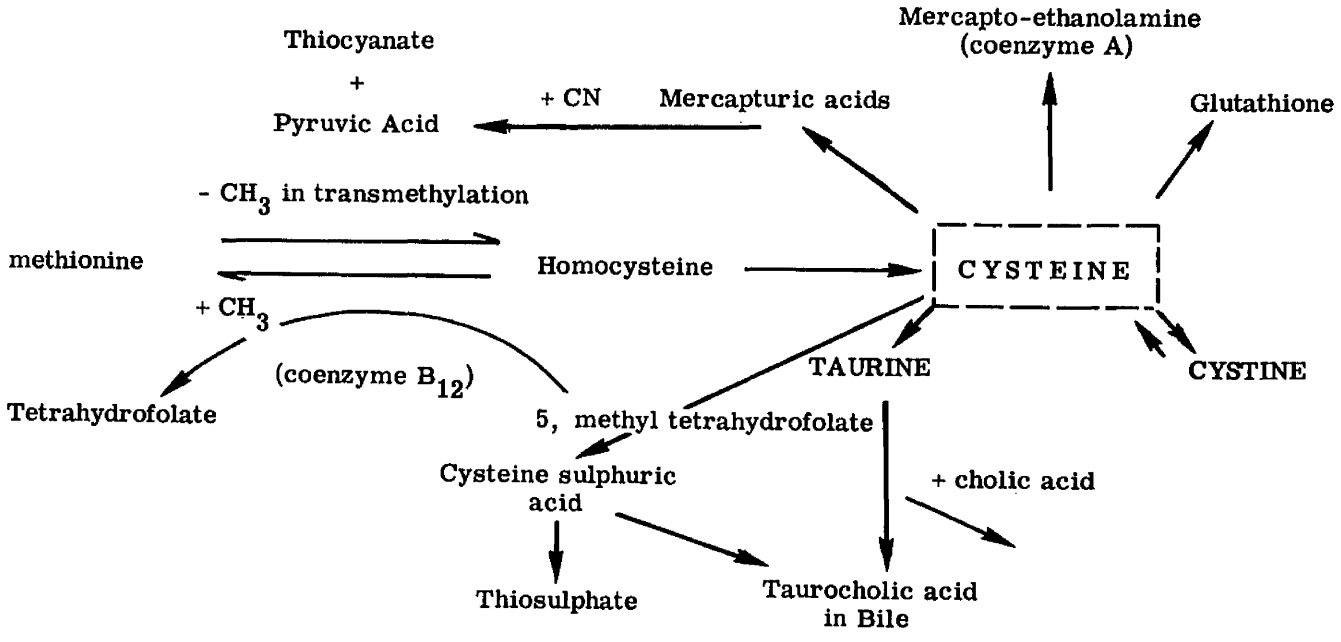


Figure 7.2. Summary of known Cysteine reactions.

are degraded to urea and sulphate. The scheme is outlined in Figure 7, 2 from which it can be seen that necessary substrates for the conversion of cyanide to thiocyanate are made available. Both coenzyme vitamin B12 and folate are necessary for the successful preparation.

Failure to detoxify cyanide may occur in several ways. Loss of liver parenchymal cells will interfere with the conversion of cyanide to thiocyanate due to diminished available rhodanese, thus explaining toxic amblyopia in patients with cirrhosis in whom the serum vitamin B12 concentration is often high.

Lack of coenzyme B12 will interfere with the conversion of methionine to cysteine and this may be a factor in cases of optic neuropathy of pernicious anaemia. Such patients would be expected to show a high concentration of folic acid in the serum, as has been shown.

Malnutrition will interfere with the preparation of a sulphur donor by dietary deficiency of vitamin B12, folic acid and the necessary proteins. Such patients will

show low concentrations of vitamin B12 and folic acid in their serum. Osuntokun et al (1969) have suggested that dietary deficiency of cystine is critical in tropical nutritional neuropathy.

Wilson (1963) has suggested that in Leber's hereditary optic atrophy there is an enzymic deficiency which prevents the conversion of cyanide to thiocyanate. If this is so, it is difficult to explain the rise in thiocyanate found in the body fluids of patients suffering from this condition on treatment with hydroxocobalamin. Although in general patients suffering from Leber's hereditary optic atrophy show normal serum concentrations of vitamin B12 and normal vitamin B12 absorption (Foulds et al 1968c), there may be a defect in the preparation of the suitable sulphur donor either before or after the point at which coenzyme B12 is utilised.

Direct combination with cyanide or replenishing coenzyme B12 stores may be functions of hydroxocobalamin, but neither explain the observed alteration in renal clearance of thiocyanate found in patients whilst on

treatment with hydroxocobalamin.

It is proposed that therapeutic hydroxocobalamin may affect the tubular resorption of thiocyanate, or alternatively the tubular epithelium may be the site of extra hepatic conversion of cyanide to thiocyanate. Such renal tubular mechanisms concerned in the active reabsorption of thiocyanate from the glomerular filtrate, or active secretion of thiocyanate into the renal tubules, may have been "poisoned" by the abnormally high concentrations of cyanide and became corrected by the removal of cyanide on therapy with hydroxocobalamin. However, the renal clearance values for thiocyanate in untreated tobacco amblyopia and Leber's hereditary optic atrophy were not abnormal when compared with the values found by Stoa (1957) in healthy smokers.

It seems more probable that the tubular epithelium of the kidney is in fact a site for the conversion of cyanide to thiocyanate. This thiocyanate partly diffuses into the blood stream and partly is secreted into the renal tubules. Such a mechanism would require not to be dependent

on rhodanese as it has been demonstrated that on chronic cyanide exposure, rhodanese activity becomes lost, (Sorbo 1951, Schievelbein et al 1969) but would be an alternative and little used pathway, present in both healthy smokers and tobacco amblyopes.

The possible source of the thiocyanate would be from the union of mercaptopyruvic acids with cyanide or the degradation of thiocyanopyruvic acid following on the oxidative deamination of B thiocyanoalanine in the kidney.

An alternative reasoning would be contained in the proposal that cyanide combines with a body constituent to form a vitamin B12 antagonist. Smith (1965) shows evidence that monocarboxylic acids act as antagonists to vitamin B12 action. The union of cyanide with cystine has been shown to produce 2-imino-4-thiazolidinecarboxylic acid, a monocarboxylic acid. This acid then blocks the coenzyme action of vitamin B12 in the formation of thiocyanate. By simply raising the amount of available coenzyme B12 this competitive inhibition is overcome.

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APPENDIX A. DETAILS of PATIENTS.

	1	2	3	4	5	6	7
AGE in YEARS	56	70	69	66	46	69	70
TOBACCO CONSUMPTION in OZS. per WK.	0.5	3.5	4.5	4.0	4.5	1.0	2.0
DURATION OF SYMPTOMS in MONTHS.	9	4	3	2	Indefinite	18	Indefinite
VISUAL ACUITY R.E. Snellen.	6/18 N36	6/36 N12	6/18 N18	6/18 N48	6/9 N5	CF N48	2/36 N36
F.M. 100 HUE R.E.	464	717	339	792	471	835	817
SERUM VITAMIN B12 in pg/ml.	25	180	118	265	248	476	46
SERUM FOLATE in mug/ml.	11.9	7.3	-	11.8	6.0	8.0	Normal
COINCIDENT DISEASE.	P.A.	NIL	NIL	NIL	-	Pre-P.A.	P.A.
TREATMENT.	Cyano 6/12 hydroxo	Hydroxo	Hydroxo	Abstained from tobacco	-	Cyano 8/12 hydroxo	Cyano 10/12 Hydroxo
FOLLOW UP PERIOD in MONTHS.	1 mgm. 30	19	35	12	default	24	36
RESULT.	+++	+++	+++	+++	-	-	

PATIENT NUMBER	8	9	10	11	12	13	14
AGE in YEARS	64	72	56	61	57	71	77
TOBACCO CONSUMPTION in OZS. per WK.	2.0	3.0	1.5	7.0	4.0	2.0	2.5
DURATION OF SYMPTOMS in MONTHS.	1	3	12	5	12	8	Indefinite
VISUAL ACUITY R.E. Snellen.	< 6/60 < N48	6/60 N24	6/18 N8	6/60 N12	6/60 N48	3/60 N24	6/18 N8
[illegible] 100 HUE R.E.	N.D	596	875	888	526	881	414
SERUM VITAMIN B12 in p./ml.	32	520	720 already on B_{12}	270	240	40	316
SERUM FOLATE in mug/ml.	N.D.	N.D.	7;7	12.0	7.5	9.5	6.9
CONCURRENT [illegible]	P.A.	Nil	Nil	Nil	Diabetes	P.A.	Nil
TREATMENT.	-	Hydroxo	Hydroxo	Hydroxo	Hydroxo	Hydroxo	Hydroxo
FOLLOW UP PERIOD in MONTHS.	Default	6	5	26	35	10	10
RESULT.	-	++	+	+++	++	++	+++

PATIENT NUMBER	15	16	17	18	19	20	21
AGE in YEARS	66	73	84	66	75	73	62
TOBACCO CONSUMPTION in OZS. per WK.	3.0	2.0	4.0	2.5	2.5	1.5	2.0
DURATION OF SYMPTOMS in MONTHS.	3	2	Poor since T.A. 1959	2 years	12	6	2
VISUAL ACUITY R.E. Snellen.	6/60 -	3/60 L.V.A.=N12	CF	1/60 N36	3/36 L.V.A.=N12	6/18 N14	6/36 -
F.M. 100 HUE R.E.	1022	1081	630	986	848	793	552
SERUM VITAMIN B12 in pg/ml.	246	352	< 25	126	70	174	250
SERUM FOLATE in mug/ml.	N.D.	3.8	8.4	6.0	N.D.	4.1	5.5
COINCIDENT DISEASE.	Nil	Cardiac failure	P.A. 2nd attack T.A.	Pre-P.A. Met.enceph.	Mac. degn.	Pre-P.A.	Nil
TREATMENT.	Abstained from tobacco	Hydroxo	Cyano up till Hydroxo	Cyano 6/12 Hydroxo	Hydroxo	Hydroxo	Abstained from tobacco
FOLLOW UP PERIOD in MONTHS.	12	9	24	30	24	Default	Default
RESULT.	+++	++	-	-	+	-	-

PATIENT NUMBER	22	23	24	25	26	27	28
AGE in YEARS	64	73	70	69	83	50	60
TOBACCO CONSUMPTION in OZS. per WK.	3.0	3.0	2.0	2.0	2.0	0.5	2.0
DURATION OF SYMPTOMS in MONTHS.	4	4	3	Indefinite	8	Indefinite	5
VISUAL ACUITY R.E. Snellen.	6/24 N8	6/24 N18	N/36 N24	6/18 N5	6/60 -	6/60 N14	2/60 N36
F.M. 100 HUE R.E.	824	757	720	362	696	392	720
SERUM VITAMIN B12 in pg/ml.	184	346	212	156	250	84	520
SERUM FOLATE in mug/ml.	N.D.	3.6	4.2	12.8	N.D.	11.6	3.8
COINCIDENT DISEASE.	Nil	Pre-P.A. Rodent ulcer	2nd attack T.A.	Glaucoma Pre-P.A. Hemicolectomy for Ca.	Nil	2nd attack T.A. P.A.	Nil
TREATMENT.	Hydroxo	Hydroxo	Hydroxo	Cyano 1/12 Hydroxo	Abstained from tobacco	Hydroxo	Hydroxo
FOLLOW UP PERIOD in MONTHS.	28	24	27	26	6	12	10
RESULT.	+++	+++	+++	+++	+	+++	+++

PATIENT NUMBER	29	30	31	32	33	34	35
AGE in YEARS	70	55	50	64	62	57	65
TOBACCO CONSUMPTION in OZS. per WK.	3.0	4.0	2.0	2.0	0.5	2.0	3.0
DURATION OF SYMPTONS in MONTHS.	6	3	5	6	12	1	8
VISUAL ACUITY R.E. Snellen.	6/18 N10	6/18 N12	6/6 N5	6/36 N12	6/36 N8	6/60 N48	6/24 -
F.M. 100 HUE R.E.	265	424	260	666	600	472	783
SERUM VITAMIN B12 in pg/ml.	< 25	400	150	360	34	160	236
SERUM FOLATE in mug/ml.	9.3	5.3	7.0	2.2	7.3	720	4.6
COINCIDENT DISEASE.	P.A.	Nil	Nil	Nil	P.A.	Cirrhosis folate Mac.anaemia	Nil
TREATMENT.	Hydroxo	Abstained from tobacco	Hydroxo	Hydroxo	Hydroxo	Hydroxo + Folate	Hydroxo
FOLLOW UP PERIOD in MONTHS.	29	12	18	5	24	5	21
RESULT.	+++	+++	+++	++	+++	+++	++

PATIENT NUMBER	36	37	38	39	40	41	42
AGE in YEARS	50	68	77	80	73	55	59
TOBACCO CONSUMPTION in OZS. per WK.	0.5	2.5	3.0	2.0	4.0	3.0	3.0
DURATION OF SYMPTOMS in MONTHS.	3	2	12	indefinite	indefinite	2	7
VISUAL ACUITY R.E. Snellen.	5/60 N24	6/36 N10	6/12 N5	6/60 N18	6/18 N6	6/36 -	6/18 N14
F.M. 100 HUE R.E.	796	408	1068	660	1090	556	849
SERUM VITAMIN B12 in pg/ml.	<20	182	306	324	316	122	248
SERUM FOLATE in mug/ml.	3.2	3.4	3.9	4.5	6.9	-	7.0
COINCIDENT DISEASE.	P.A.	Cl.osteo myelitis	Nil	Nil	-	Cirrhosis	Diabetes
TREATMENT.	Cyano hydroxo	Hydroxo	Cyano 5/12 hydroxo	Hydroxo	Hydroxo	Cyano 10/12 hydroxo	Cyano 1/12 hydroxo
FOLLOW UP PERIOD in MONTHS.	25	20	29	9	default	died	31
RESULT.	+++	+++	+++	+	-	-	+++

	42	44	45	46	47	48	49
AGE in YEARS	62	71	68	68	56	75	83
TOBACCO CONSUMPTION in OZS. per WK.	3.0	3.5	3.5	2.0	3.0	3.0	3.5
DURATION OF SYMPTOMS in MONTHS.	9	12	4	4	4	4	3
VISUAL ACUITY R.E. Snellen.	6/36 N18	6/60 -	6/60 N14	3/60 N36	6/24 -	4/60 N48	1/60 N24
F.M. 100 HUE R.E.	1159	890	650	464	-	1120	904
SERUM VITAMIN B12 in pg/ml.	350	230	410	310	456	128	140
SERUM FOLATE in mug/ml.	4.5	6.1	4.0	-	-	3.9	-
COINCIDENT DISEASE.	Hypertension Pre-P.A.		Nil	Pre-P.A.	-	Pre-P.A.	2nd attack T.A.
TREATMENT.	Hydroxo	Hydroxo	Cyano 2/52 Hydroxo	Hydroxo	-	Cyano 4/12 Hydroxo	Hydroxo
FOLLOW UP PERIOD in MONTHS.	8	25	22	15	Default	30	10
RESULT.	++	+	+++	+++	-	-	-

	50	51	52	53	54	55	56
AGE in YEARS	71	53	63	78	49	72	73
TOBACCO CONSUMPTION in OZS. per WK.	2.0	5.0	4.0	2.5	1.5	2,5	3.5
DURATION OF SYMPTOMS in MONTHS.	12	1	2	3	3	5	12
VISUAL ACUITY R.E. Snellen.	6/36 N18	6-/36 N48	3/60 N48	6/36 -	6/24 N18	3/36 N36	6/60 N18
F.M. 100 HUE R.E.	1290	901	826	701	676	1142	683
SERUM VITAMIN B12 in pg/ml.	110	490	186	113	142	318	148
SERUM FOLATE in mug/ml.	4.6	8.7	7.6	1.4	5.6	18.0	N.D.
COINCIDENT DISEASE.	Surgery for peptic ulcer P.A.	Takes systemic steroid for bronchitis	2nd attack Tobacco amb.	Nil	Diabetes	Diabetes	Pre- P.A.
TREATMENT.	Hydroxo	Hydroxo	Hydroxo	Hydroxo	Hydroxo	Hydroxo	Hydroxo
FOLLOW UP PERIOD in MONTHS.	8	5	20	21	29	27	Defaulted but v.a. improving
RESULT.	+	0	-	+++	+++	++	-

PATIENT NUMBER	57	58	59	60	61	62	63
AGE in YEARS	73	64	59	67	79	67	76
TOBACCO CONSUMPTION in OZS. per WK.	0.5	3.0	5.0	2.5	1.0	4.0	4.0
DURATION OF SYMPTOMS in MONTHS.	2	9	2	3	2	3	18
VISUAL ACUITY R.E. Snellen.	6/18 N12	6/24 N24	6/24 N24	6/60 N36	6/60 N36	3/60 N24	6/24 N8
F.M. 100 HUE R.E.	891	536	623	732	999	697	780
SERUM VITAMIN B12 in pg/ml.	470	102	204	360	554	435	320
SERUM FOLATE in mug/ml.	8.8	5.2	7.6	4.6	6.0	6.6 systemic steroid	2.7
COINCIDENT DISEASE.	2nd attack T.A. P.A.	Pre P.A.	Glaucoma	Padget's disease	Diabetes	Rheumatoid arthritis. Congenital Dichromat.	Colectomy for Ca. Pre P.A.
TREATMENT.	Hydroxo	Hydroxo	Cyano 2/12 Hydroxo 12/12 Abstain	Hydroxo	Hydroxo	Hydroxo	Hydroxo
FOLLOW UP PERIOD in MONTHS.	8	33	28	18	default	10	15
RESULT.	-	+++	+++	+++	-	++	-

APPENDIX B. Publications.

1. Cyanocobalamin versus Hydroxocobalamin in the treatment of Tobacco Amblyopia, Lancet, 1967, 2,450.

2. Hydroxocobalamin in the treatment of Leber's hereditary optic atrophy, Lancet 1968, 1, 896.

3. Serum Thiocyanate Concentrations in Tobacco Amblyopia, Nature, 1968, 218, 586.

4. Response of Metabolic encephalopathy to treatment with Cyanocobalamin, Scot. Med.J., 1968, 13,274

5. Vitamin B12 absorption in Tobacco Amblyopia, Brit. J. Ophthal., 33,393

PATIENT NUMBER	64	65					
AGE in YEARS	74	77					
TOBACCO CONSUMPTION in OZS. per WK.	2.0	3.0					
DURATION OF SYMPTOMS in MONTHS.	24	3					
VISUAL ACUITY R.E. Snellen.	2/60	6/24					
F.M. 100 HUE R.E.	1050	798					
SERUM VITAMIN B12 in pg/ml.	110	572					
SERUM FOLATE in mug/ml.	5.4	5.5					
COINCIDENT DISEASE.	Diabetes	Nil					
TREATMENT.	Hydroxo 1 mgm.	Hydroxo					
FOLLOW UP PERIOD in MONTHS.	16	defaulted					
RESULT.	++	-					